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EPIDEMIOLOGICAL, CLINICAL, AND PROGNOSTIC FACTORS IN ADULT-TYPE OVARIAN GRANULOSA CELL TUMORS

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ACADEMIC DISSERTATION

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To my family

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Abstract

Adult-type granulosa cell tumors (AGCTs) belong to the sex cord-stromal group of ovarian tumors and account for 3-5% of ovarian neoplasms. Etiological factors of AGCTs remain mostly unknown, although studies have found a somatic missense mutation in the transcription factor *FOXL2*. AGCTs are usually diagnosed at an early stage and have a favorable prognosis compared with the more common epithelial ovarian cancer. However, tumor recurrence develops in up to 35% of patients, even in early-stage disease - often unpredictably and several years or even decades after the primary diagnosis. Therefore, the clinical picture of AGCT is rather different from that of other subtypes of ovarian cancer.

The aims of this study were to determine the incidence and epidemiological background of AGCTs in a large, multinational Nordic cohort and to estimate the incidence of other, especially endocrine-related primary malignancies among patients with AGCT. Furthermore, the objective was to describe the clinical characteristics and prognostic factors linked to AGCT–related recurrence and survival, and to introduce an optimal follow-up strategy for these patients.

The international incidence rate of AGCT has varied between 0.47/100,000 and 1.6/100,000. Our epidemiological registry study on AGCT incidence utilized the Finnish, Icelandic, Norwegian, and Swedish Cancer Registry data on AGCTs over several decades. We showed that the age-adjusted incidence rates were quite constant in 1953-2012: about 0.6-0.8 per 100,000 for most of the study period. The age-specific incidence was highest at 50-64 years of age, and there were no occupations with significantly increased risk of AGCT. The conclusions drawn from these results point to AGCT as a primarily sporadic, not exposure-related cancer, typically occurring in peri- or postmenopause.

We estimated the incidence of other primary malignancies among AGCT patients using data from the Finnish Cancer Registry in 1968-2013. After AGCT, we found increased risks for cancers of the soft tissue and thyroid as well as leukemia, which likely indicate shared risk factors and therapy-induced side effects. The incidence of AGCT was significantly increased among women with previous breast cancer, suggesting shared hormonal etiology or treatment-induced effects.

To evaluate the clinical and prognostic factors, all AGCT patients diagnosed at Helsinki University Hospital (HUH) during nearly six decades were included in the clinical study cohort (n=240). The mean follow-up in these studies was over 15 years. After a histological review, we analyzed the clinical data for association with both AGCT-related and overall survival and tumor relapse. Of the original study cohort, the diagnosis was first histologically confirmed in 78% of patients and then molecularly confirmed for the *FOXL2* mutation in 68% of patients.

In multivariate analysis, stage was the only independent prognostic factor related to AGCT-specific survival. Spontaneous or iatrogenic tumor rupture was independently associated with tumor recurrence.

By utilizing the extensive cancer registry data together with the internationally unique, large and carefully validated single-institute patient cohort, these studies reveal the diagnostic challenges of AGCTs, and provide novel epidemiological data and evidence-based tools to develop follow-up strategies for this rare cancer.

Tiivistelmä

Aikuistyypin munasarjan granuloosasolukasvain (AGSK) kuuluu sukupienakasvaimiin ja käsittää 3-5 % kaikista munasarjasyövistä. Kasvaimen etiologia tunnetaan huonosti, mutta sille on osoitettu somaattinen pistemutaatio transkriptiotekijä *FOXL2*:a koodaavassa geenissä. AGSK todetaan yleensä varhaisvaiheessa, ja sen ennuste on hyvä verrattuna yleisempään epiteliaaliseen munasarjasyöpään. Kuitenkin jopa 35 %:lla potilaista kasvain uusiutuu ennustamattomasti, usein vuosia tai joskus jopa vuosikymmeniä taudin toteamisen ja hoidon jälkeen. Näin ollen AGSK:n taudinkuva eroaa melko paljon muista munasarjasyövän alatyypeistä.

Tämän tutkimuksen tarkoituksena oli tutkia AGSK:n kansainvälistä esiintyvyyttä ja epidemiologista taustaa laajassa pohjoismaisessa aineistossa, sekä selvittää muiden, erityisesti hormoniriippuvaisten syöpien esiintyvyyttä kyseisillä potilailla. Lisäksi pyrkimyksenä oli määrittää AGSK:n kliinistä taudinkuvaa ja ennustetekijöitä sekä taudin uusiutumisen että tautispesifin kuolleisuuden suhteen. Tulosten avulla pyrimme muodostamaan näille potilaille optimaalisen seurantastrategian kasvaimen toteamisen jälkeen.

Kansainvälisissä julkaisussa AGSK:n raportoitu insidenssi on vaihdellut välillä 0,47-1,6/100 000. Oma kattava rekisteritutkimuksemme hyödynsi Suomen, Ruotsin, Norjan ja Islannin syöpärekisteritietoja usean vuosikymmenen ajalta. Ikäkorjattu insidenssi pysyi vuosina 1953-2012 melko tasaisena, noin 0,6-0,8/100 000. Ikäryhmittäisessä analyysissa AGSK:n insidenssi oli korkeimmillaan 50-64-vuotiaiden ryhmissä, eikä erityisiä ammattialtisteita löydetty eri ammattiryhmiä tutkittaessa. Johtopäätöksenä tuloksista voidaan todeta, että AGSK on pikemmin satunnaisesti ilmaantuva kuin altistesidonnainen syöpä, joka ilmenee tyypillisesti vaihdevuosi-iässä tai tämän jälkeen.

AGSK-potilailla ilmeneviä muita syöpiä tutkittiin käyttämällä Suomen Syöpärekisterin aineistoa vuosilta 1968-2013. AGSK:n jälkeen todettiin tilastollisesti merkitsevästi enemmän pehmytkudos- ja kilpirauhassyöpää sekä leukemiaa, mikä todennäköisesti viittaa yhteisiin riskitekijöihin sekä primaarisyövän hoitojen myöhäisiin haittavaikutuksiin. Rintasyövän sairastaneilla naisilla esiintyi merkitsevästi enemmän AGSK:ta, mikä saattaa viitata näille kasvaimille yhteiseen hormonaaliseen etiologiaan tai rintasyövän hoitojen vaikutukseen.

Kliiniseen aineistotutkimukseen otettiin mukaan kaikki HYKS (Helsingin Yliopistollinen Keskussairaala) Naistenklinikalla hoidetut AGSK-potilaat lähes kuuden vuosikymmenen ajalta (N=240). Kasvaimien histologinen diagnoosi varmistettiin ja aineisto analysoitiin sekä kokonais- ja tautispesifin kuolleisuuden että kasvaimen uusiutumisen suhteen. Alkuperäisestä potilasaineistosta diagnoosi varmistui histologisessa uudelleenarviossa 78 %:ssa tapauksista, ja molekulaarisesti *FOXL2*-mutaation suhteen 68 %:ssa tapauksista.

Monimuuttuja-analyyseissa kasvaimen levinneisyys (stage) toteamishetkellä oli ainoa itsenäinen ennustetekijä tautispesifin kuolleisuuden suhteen, mutta kasvaimen puhkeaminen joko spontaanisti tai leikkauksen aikana ennusti itsenäisesti AGSK:n uusiutumista.

Nämä kansainvälisesti ainutlaatuisen laajan ja tarkkaan vahvistetun potilasaineiston sekä kattavan syöpärekisteriaineiston avulla tehdyt tutkimukset osoittavat AGSK:n diagnostiset haasteet ja tuovat tämän harvinaisen syövän osalta sekä uutta epidemiologista tietoa että välineitä kehittää näyttöön perustuvia seurantastrategioita.

List of original publications

This thesis is based on the following publications:

I Bryk S, Pukkala E, Martinsen J-I, Unkila-Kallio L, Tryggvadottir L, Sparén P, Kjaerheim K, Weiderpass E, Riska A. Incidence and occupational variation of ovarian granulosa cell tumours in Finland, Iceland, Norway, and Sweden during 1953-2012: a longitudinal cohort study. BJOG 2017 Jan;124(1):143-149.

II Bryk S, Pukkala E, Färkkilä A, Heikinheimo M, Unkila-Kallio L, Riska A. Other primary malignancies among women with adult-type ovarian granulosa cell tumors. Submitted.

III Bryk S, Färkkilä A, Bützow R, Anttonen M, Heikinheimo M, Leminen A, Riska A, Unkila-Kallio L. Clinical characteristics and survival of patients with an adult-type ovarian granulosa cell tumor: a 56-year single-center experience. Int J Gynecol Cancer 2015 Jan;25(1):33-41

IV Bryk S, Färkkilä A, Bützow R, Leminen A, Tapper J, Heikinheimo M, Unkila-Kallio L, Riska A. Characteristics and outcome of recurrence in molecularly defined adult-type ovarian granulosa cell tumors. Gynecol Oncol 2016 Dec;143(3):571-577

Abbreviations

AGCT	adult-type granulosa cell tumor
AMH	anti-Müllerian hormone
BEP	bleomycin/etoposide/cisplatin
BMI	body mass index
BRCA1/BRCA2	breast cancer 1/breast cancer 2
CA12-5	cancer antigen 125
CI	confidence interval
CT	computerized tomography
E2	estradiol
ESMO	European Society for Medical Oncology
FATWO	female adnexal tumor of probable Wolffian origin
FCR	Finnish Cancer Registry
FDG-PET/CT	F-18 fluorodeoxyglucose positron emission tomography/computerized tomography
FIGO	International Federation of Gynecology and Obstetrics
FIN-GOG	Finnish Society of Gynecologic Oncology
FOXL2	forkhead box L2
FSH	follicle stimulating hormone
GCT	granulosa cell tumor
GnRH	gonadotropin-releasing hormone
HE4	human epididymis protein 4
HRT	hormone replacement therapy
HUH	Helsinki University Hospital
IARC	International Agency for Research on Cancer
ICD-O-3	The International Classification of Diseases for Oncology, 3rd Edition
MI	mitotic index
MRI	magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
NOCCA	Nordic Occupational Cancer Study
ROC	receiver operating characteristic
RT	radiotherapy
SCST	sex cord-stromal tumor
SCTAT	sex cord tumor with annular tubules
SEER	Surveillance, Epidemiology, and End Results Program
SERM	selective estrogen receptor modulator
SIR	standardized incidence ratio
SPM	second primary malignancy

1 Introduction

With 422 new cases in Finnish women in 2014, ovarian cancer was the 11th most common cancer and the fifth most common cause of cancer death (www.cancer.fi). Despite major changes in lifestyle, diagnostics and treatment in recent decades, both the age-adjusted incidence and the age-adjusted mortality rates of ovarian cancer have remained quite stable over the past 50 years.

Adult-type granulosa cell tumor (hereafter referred to as AGCT) is a rare subtype of ovarian cancer, which constitutes the vast majority of sex cord-stromal tumors (SCSTs). They arise from the granulosa cell of the preovulatory follicle and are hormonally active. AGCTs are known to produce estradiol (E2), inhibins, and anti-Müllerian hormone (AMH). Partly due to hormone secretion, they are usually diagnosed at an early stage and have a relatively good prognosis with a 10-year survival rate of over 90%. However, frequent yet long delays to tumor recurrence are typical of AGCT, leading to increased disease-related mortality.

AGCT is characterized by a single somatic point mutation in the transcription factor *FOXL2* (402C->G), which was discovered by Shah et al. in 2009. The *FOXL2* mutation is pathognomonic to AGCT and can be used in differential diagnostics. The histological diagnosis of AGCT is challenging, with the rate of false-positive diagnosis reaching 40% in some series. Most clinical studies on AGCT lack the diagnostic validation with *FOXL2* mutation status, and little is still known about the etiology or international incidence of AGCTs. Furthermore, the unexpectedly recurring tumors present a major clinical challenge in both treatment and follow-up. No prognostic markers exist to identify patients at increased risk of recurrence, and new tools to monitor these high-risk patients are needed.

As AGCT is a hormone-producing cancer, there is a possibility of an increased risk for other endocrine-related malignancies, such as endometrial and breast cancer, among these patients. The typically long and indolent disease course of AGCT calls for novel epidemiological evidence on the risk of other, especially estrogen-related, primary malignancies. Also, no previous studies on occupational risks for AGCT exist.

This study reports unique AGCT incidence data from several Nordic countries, as well as one of the largest series to date of histologically and/or molecularly validated AGCT patients treated in a single institute over a six-decade follow-up period. The study aimed to provide up-to-date and novel data on the epidemiology and clinical and prognostic factors of AGCTs.

2 Review of the literature

Ovarian cancer includes three subtypes based on the cell types from which they arise in the ovary (1) (Figure 1). The majority of these cancers are ovarian carcinomas, i.e. epithelial tumors (2, 3). These account for approximately 90% of all ovarian cancers. The second largest group (5-10%) are the sex cord-stromal tumors (SCSTs) (4). The third and smallest group are germ cell tumors (1-2%) (5).

Of all sex cord-stromal tumors, granulosa cell tumors (GCTs) are the most common (90%) (4, 5). GCTs can be further classified into adult and juvenile types, of which adult-type tumors (AGCTs) comprise 95% (6). They exhibit similar morphological and biochemical features to normal proliferating granulosa cells of the late preovulatory follicle (5). Juvenile GCTs display distinct clinical, histological, and molecular features, and typically occur in prepubertal girls or young women, often presenting with precocious puberty (6). Other SCSTs include Sertoli-Leydig cell tumors, fibromas, thecomas, steroid cell tumors, sex cord tumors with annular tubules (SCTATs), and mixed forms, including gynandroblastomas (7). The present study focuses on AGCT, the adult-type granulosa cell tumor, and describes the unique features of this cancer subtype, often in comparison to epithelial ovarian cancer, or ovarian cancer in general.

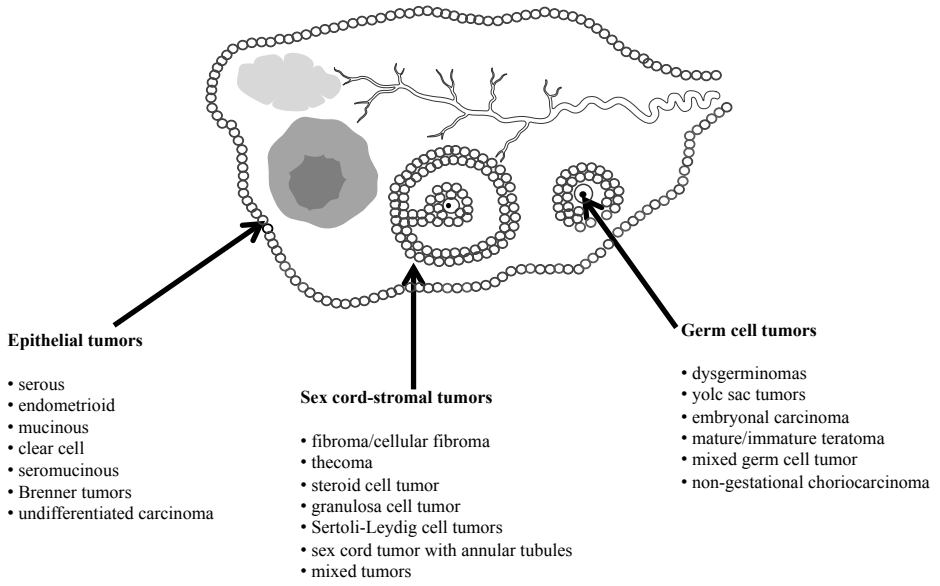

Figure 1. *Origins of different subtypes of ovarian cancer. Modified from Chen et al. (1).*

2.1 Epidemiology of granulosa cell tumors

2.1.1 Incidence

According to the World Cancer Report, there are about 239,000 new cases of ovarian cancer annually worldwide (8), and thus, the estimated annual number of AGCTs is approximately 10,000. The global incidence of AGCT is unknown, but the reported incidence in the Netherlands, Sweden, Israel, Denmark, and Finland ranges from 0.5 to 1.4/100,000, and the estimated incidence in the US is 0.99/100,000 (6, 9-13).

2.1.2 Etiology and risk factors

Pathogenesis and FOXL2

The pathogenesis of AGCT is still largely unexplained, but a single somatic missense mutation in the transcription factor *FOXL2* (402 C-G) is pathognomonic to these tumors, and the mechanism for how the mutation causes tumor formation is an area of active investigation (14-17). *FOXL2* belongs to the forkhead box (FOX) family of transcription factors and plays a fundamental role in ovarian development and maintenance, e.g. in formation of follicles and differentiation of granulosa cells (17). It has been suggested to act as an oncogene or tumor suppressor gene in AGCT (4, 17). The main effect of the mutation seems to be in increased proliferation, decreased apoptosis, and hormonal alterations (14). The *FOXL2* C134W mutation is unique to AGCT, and it is not found in juvenile GCTs or other tumor types (4). The presence of the *FOXL2* mutation, therefore, also provides a molecular diagnosis of AGCT. In contrast to epithelial ovarian cancer, the karyotype of AGCTs is relatively stable (4).

Hereditary risk factors

There is no known genetic predisposition for AGCT specifically, but in Peutz-Jeghers syndrome, a rare autosomal dominant disorder, there is an increased risk for an intermediate sex cord-stromal tumor between AGCT and Sertoli cell tumors (4, 5, 18). Olliers disease and Maffucci syndrome are rare inherited disorders that are associated with juvenile GCTs, and in DICER1 syndrome sex cord-stromal tumors, predominantly Sertoli-Leydig cell tumors, are found (4). One case report of AGCT in two first-degree relatives has been published; this is most likely a coincidental finding (19). *BRCA1* and *BRCA2* mutations, which predispose to epithelial ovarian cancer, are not associated with the development of AGCT (6).

Reproductive and hormonal factors

Although AGCT can present at any age, it typically occurs around menopause, suggesting that the perimenopausal hormonal changes contribute to its pathogenesis (18). As a whole, early age of menarche, late age of menopause, nulliparity, infertility, obesity, and a family history of ovarian cancer increase the risk for ovarian cancer, whereas high parity, use of oral contraceptives, hysterectomy, and tubal ligation reduce this risk (20-25). Current evidence indicates that hormone replacement therapy (HRT), regardless of type or regimen, increases the risk for serous and possibly endometrioid cancer, but not mucinous ovarian cancer (26-28). This risk seems to be evident at least after five years, possible even less than five years of HRT use (27, 28). The relationship between HRT use and development of AGCT remains to be elucidated.

Overall, the risk factors for non-epithelial ovarian cancer seem to differ from those of epithelial cancer (29, 30). However, the protective roles of oral contraceptives and high parity also in AGCT or stromal cancer development have been suggested (20, 31). The effect of ovulation inducers, such as clomifene citrate and selective estrogen receptor modulators (SERMs), on AGCT pathogenesis has been discussed, but no evidence has yet shown causality between these factors (5, 12, 32). Infertility as such may constitute a risk factor for developing ovarian cancer, and current evidence does not support an association between the use of ovulation-inducing drugs and ovarian cancer in general (33, 34). A recent Swedish study proposed an association between preterm birth and the development of sex cord-stromal tumors, and another study between elevated serum androgens and the development of SCSTs (35, 36).

Environment and ethnicity

The rates of ovarian cancer vary between geographical regions, with the highest reported incidence rates in northern and eastern Europe, North America, and Oceania (8). In immigration studies, the rates of ovarian cancer in second-generation immigrants approach those in native residents, indicating that environmental factors influence tumor development (37, 38). However, there are also significant differences in ovarian cancer incidence among different ethnic groups, with populations of European/Asian origin typically having higher risks than those of African descent (25, 39).

In the few studies focusing on the epidemiology of AGCT, the results are somewhat contradictory. Ohel et al. (1983) found the risk of developing AGCT to be almost twice as high in women of European/American descent than in those of Asian/African origin (11), whereas Boyce et al. (2009) reported significantly more non-whites among AGCT patients in a case-control study comparing AGCT patients with the general population and with patients with epithelial ovarian cancer (31). Additionally, this study found the risk of AGCT to be higher for obese women, and decreased in women who smoked, which was hypothesized to result from effects of increased or decreased estrogen, similarly to endometrial cancer (31). A study based on the US Surveillance, Epidemiology, and End

Results Program (SEER) concluded that white women had significantly higher rates of epithelial tumors than black women, whereas the opposite was true for gonadal stromal tumors (39).

2.1.3 Occupational risks of ovarian cancer

Associations between occupations or exposures and cancer vary among cancer types, with the strongest causality shown between mesotheliomas and asbestos exposure (40). A rather recent meta-analysis also found an association between asbestos exposure and ovarian cancer (41). However, the risk of cancer in certain occupations is not only explained by direct carcinogenic exposure, but also on the person's social environment. Direct occupational hazards are thought to only partly explain the observed variation in cancer incidence among different occupations, while the effects of lifestyle factors such as longer education and decreasing physical activity are increasing (40). Studies on occupational risks are typically limited by the absence of confounding factors such as, in the case of ovarian cancer, reproductive characteristics or use of hormonal therapies, or lack of specific exposure information. Nevertheless, the results of these studies may assist in identifying previously unknown etiological factors and guide in cancer prevention.

Studies on occupational risks specific for ovarian cancer are limited, and no previous studies on occupational risk factors regarding AGCTs exist. A number of studies have showed an increased risk for ovarian cancer among nurses (42-46), teachers (44, 45), and hairdressers/beauticians (40, 47-50) (Table 1). In nurses, this is thought at least partly to reflect the amount of shift and/or night work involved (22). Shift work resulting in circadian disruption has been classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC) (51). The effect is likely to be mediated by the pineal gland hormone melatonin, which in turn is hypothesized to affect reproductive hormones, thus increasing the risk of ovarian cancer (52). Additionally, the elevated risk for ovarian cancer among nurses and teachers is often explained by confounding factors such as low parity (53), but Le et al. (2014) demonstrated a significantly elevated risk for epithelial ovarian cancer in teaching occupations after adjustment for potential confounders including body mass index (BMI), oral contraceptive use, menopausal hormonal therapy, parity, age at menarche, first childbirth, menopause, family history of breast and ovarian cancer in mother and sister(s), tubal ligation, partial oophorectomy, and hysterectomy (22). Nonetheless, the explanation for this elevated risk remains unclear. Conversely, the large Nordic Occupational Cancer Study (NOCCA) did not detect any increased risk for ovarian cancer among teachers or nurses, with over 2,000 cases of ovarian cancer among both occupational categories, including assistant nurses (40). In this analysis, the risk for ovarian cancer was slightly increased among printers and hairdressers, but the overall occupational variation in ovarian cancer incidence was small.

A number of specific carcinogenic agents in ovarian cancer have been suggested, which are mainly associated with occupations in the printing and shoe or textile industry (Table 1) (47, 54-56). The evidence regarding these is, however, sparse, and a recent review concluded that asbestos is the only specific occupational exposure with a significant association with ovarian cancer, supported by the IARC classification (57).

Table 1. *Occupational risks for ovarian cancer according to previous studies.*

Occupation or industry	Exposure	Source of data
Nurses and physicians	Shift work/radiation exposure/unknown	Lie et al. 2007, MacArthur et al. 2007, Petralia et al. 1999, Sala et al. 1998, King et al. 1994
Teachers	Unknown	Le et al. 2014, MacArthur et al. 2007, Sala et al. 1998, King et al. 1994
Hairdressers/beauticians	Chemical exposure (aromatic amines, metal compounds, benzene, alcohol, talc)	Pukkala et al. 2009, Vasama-Neuvonen et al. 1999, Boffetta et al. 1994, Spinelli et al. 1984, Teta et al. 1984
Healthcare, food preparation and service, office and administrative support	Shift work	Bhatti et al. 2013, Sala et al. 1998
Accountants, bookkeepers	Sedentary work	Le et al. 2014, Sala et al. 1998
Typesetters, typographers, printers, lithographers	Chemical exposure (lead, benzene, toluene, xylene, oil mist, gasoline, printing inks and pigments, mineral oils)	Pukkala et al. 2009, Shields et al. 2002, Vasama-Neuvonen et al. 1999, Sala et al. 1998
Rubber product workers, printers, upholsterers, other graphics occupations	Chemical exposure (aliphatic, alicyclic, and aromatic hydrocarbon solvents)	Shields et al. 2002, Vasama-Neuvonen et al. 1999
Textile or shoe manufacturing, building/construction workers	Chemical exposure (silica dust, asbestos, leather dust)	Wernli et al. 2008, Camargo et al. 2011, Charbotel et al. 2014, Vasama-Neuvonen et al. 1999
Drivers, car greasers, miners	Chemical exposure (diesel exhaust)	Guo et al. 2004, Vasama-Neuvonen et al. 1999

2.1.4 Second primary cancers after ovarian cancer

As survival rates in cancer increase, it has become relevant to evaluate the long-term effects of cancer and its treatment, including the risk for another primary malignancy after the first cancer. The number of patients with multiple primary tumors has grown, with second- or higher order primary cancers accounting for 18% of incident cancers in the recent SEER program, overriding the first primary breast, lung, and prostate cancer (58).

The incidence of second primary cancer may be affected by 1) increased surveillance after primary tumor, 2) shared genetic factors, 3) lifestyle-related environmental factors, 4) treatment-induced factors, or 5) a combination of these, such as gene-environment interactions (58-60). Several studies have addressed the issue of second primary malignancy (SPM) after ovarian cancer of any type, or after epithelial ovarian cancer (60-66). In the majority of these, an increased overall risk for SPM has been demonstrated (59, 60, 62, 63, 65, 66). It has been suggested that a high incidence of synchronous cancer as well as surveillance bias may affect the estimates of SPM among these patients (60). A recent study by Hung et al (2015) reported significantly increased standardized incidence ratios (SIRs) for cancers of the colon, rectum, anus, lung, mediastinum, breast, cervix, uterus, bladder, and thyroid as well as for leukemia, after exclusion of SPM occurring within one year of ovarian cancer (60). Moreover, age equal to or over 50 years, radiotherapy, and chemotherapy were independent risk factors for SPM. Although excluded from the final analysis, the SIR for SPM was highest during the first year from primary cancer diagnosis, highlighting the importance of detecting synchronous tumors in patients with ovarian cancer. After histological re-evaluation of Swedish Cancer Registry data, Bergfeldt et al. (2000) concluded that the risk for SPM after ovarian cancer is overestimated; however also their study demonstrated increased SIRs for cancers of the gastrointestinal tract, breast, uterus, bladder, and most notably leukemia among ovarian cancer patients even after adjusting for incorrect diagnoses (64).

The explanations for SPMs after ovarian cancer vary according to tumor type. The inherent genetic factors associated with ovarian cancer are considered mainly to affect the development of breast and colorectal cancer, most importantly in *BRCA1* and *BRCA2* mutations and Lynch syndrome (58, 67, 68). Furthermore, gastrointestinal, breast, and gynecological cancers may share lifestyle-related risk factors such as obesity (68, 69).

The therapeutic agents used in the treatment of ovarian cancer may be carcinogenic, as both chemotherapy and radiotherapy can induce e.g. acute leukemia (70). The strongest associations in treatment-related SPMs are those between radiotherapy (RT) and solid tumors, and between chemotherapy and leukemia (70). The majority of RT-related solid tumors arise within the irradiated field, with a long latency of at least 5-10 years (58). In chemotherapy, several agents have been linked to myeloid neoplasms: alkylating agents, topoisomerase II inhibitors, and antimetabolites (58). Shimada et al. (2014) reported a low incidence of secondary leukemia after changing from alkylating agents to paclitaxel-based chemotherapy in ovarian cancer, indicating that developments in treatment regimens in the

past decades may change the treatment-related incidence of SPM (61). Hung et al. (2015) studied the risk of SPM for several chemotherapeutic agents and found only the use of fluorouracil to be an independent risk factor in multivariate analysis (60).

Specific studies on SPMs after AGCT are sparse (9, 71). AGCTs are typically characterized by slow and indolent growth when diagnosis is made at an early stage, leading to long survival after primary treatment (Table 2 and 3). The 10-year survival rates in AGCT have reached over 90% in recent studies (Table 4). Moreover, AGCTs are distinct cancers in their ability to produce hormones such as estrogen and inhibins (5). An estimated 70% of AGCTs produce estrogen, leading to an increased and well-known risk of concomitant endometrial pathology and endometrial cancer (5, 6, 10, 18, 72). These characteristics of AGCTs result in a clinically relevant risk of second primary cancer. In a study including 936 cases of granulosa and theca cell tumors Björkholm et al. (1980) described an increased risk of developing endometrial carcinoma and malignant lymphoma after these neoplasms, and if concomitant cancers were taken into account, also the number of breast, colon, and thyroid carcinoma were higher than expected (9). In this series, 45 of 62 cancers diagnosed at the same time with the ovarian tumor were endometrial carcinomas. In other studies, the rate of concomitant endometrial cancer with AGCT has varied from 5% to 13% (6, 10, 11, 73, 74). Endometrial carcinoma in association with AGCT is usually well-differentiated, early stage, and has a favorable prognosis (6).

Hammer et al. (2013) reported an increased risk of breast cancer in women with AGCT, when breast cancers also before the diagnosis of AGCT were included (71). Evans et al. (1980) found a 5.5% rate of breast cancer in a patient cohort including both GCTs and thecomas, and Ohel et al. (1983) described a breast cancer rate of 6.4% in a study with 172 GCTs (11, 74). In a more recent study, Meisel et al. (2015) reported more breast cancers than expected before the diagnosis of AGCT (75).

2.2 Clinical presentation

2.2.1 Symptoms and diagnosis

AGCT presents most often during the menopausal or early postmenopausal period at a median age of 50-54 years (5, 6), but the range varies from 17 to 87 years (76, 77). AGCTs in children have been reported, but this is an exceptionally rare finding, and older studies including very young patients are likely to include juvenile GCTs (5, 11, 14, 74, 78). Typical symptoms include those related to estrogen secretion such as menstrual disturbances and postmenopausal bleeding, as well as those related to mechanical distension such as bloating and abdominal pain, as AGCTs are often large and

hemorrhagic tumors (5, 6, 14). The vascularity of AGCTs may occasionally lead to tumor rupture, with intra-abdominal hemorrhage and acute pelvic pain (6, 14). However, AGCTs may also be asymptomatic and discovered in routine examination (76, 79). Tumor-derived estrogen potentially induces endometrial pathology ranging from hyperplasia to endometrial carcinoma (5, 6). Partly due to hormone secretion, AGCTs are usually diagnosed at an early stage, i.e. confined to one ovary (18, 79) (Table 2 and 3). Case reports have identified extraovarian primary AGCTs, notably in the retroperitoneum, but these are extremely rare (80-82); two out of three of these cases had a history of previous ovarian surgery for benign indications (81, 82).

Table 2. *The 2014 International Federation of Gynecology and Obstetrics (FIGO) criteria for ovarian cancer staging.*

Stage I: tumor confined to ovaries	
IA	Tumor limited to one ovary, capsule intact, no tumor on surface, negative washings
IB	Tumor involves both ovaries otherwise like IA
IC	
IC1	Surgical spill
IC2	Capsule rupture before surgery or tumor on ovarian surface
IC3	Malignant cells in the ascites or peritoneal washings
Stage II: tumor involves one or both ovaries with pelvic extension	
IIA	Extension and/or implant on uterus and/or Fallopian tubes
IIB	Extension to other pelvic intraperitoneal tissues
Stage III: tumor involves one or both ovaries with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes	
IIIA	Positive retroperitoneal lymph nodes and/or microscopic metastasis beyond the pelvis
IIIA1	Positive retroperitoneal lymph nodes only
IIIA2	Microscopic, extrapelvic (above the brim) peritoneal involvement ± positive retroperitoneal lymph nodes
IIIB	Macroscopic, extrapelvic, peritoneal metastasis ≤ 2 cm ± positive retroperitoneal lymph nodes. Includes extension to capsule of liver/spleen
IIIC	Macroscopic, extrapelvic, peritoneal metastasis > 2 cm ± positive retroperitoneal lymph nodes. Includes extension to capsule of liver/spleen
Stage IV: distant metastasis excluding peritoneal metastasis	
IVA	Pleural effusion with positive cytology
IVB	Hepatic and/or splenic parenchymal metastasis, metastasis to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)

Table 3. *The distribution (%) of clinical FIGO stages (I-IV) at initial diagnosis of GCT (series with > 100 patients)*

Study	N[1]	Stage I	Stage II	Stage III	Stage IV
Karalok et al. 2016	158	90%	1%	7%	2%
Lauszus et al. 2014	163	94%	3%	2%	1%
Van Meurs et al. 2014	127	76%	NA	NA	NA
Ertas et al. 2014	108	85%	6%	9%	0%
Suri et al. 2013	104	95%	1%	4%	0%
Sun et al. 2012	157	87%	6%	6%	1%
Lee et al. 2011	102	84%	11%	5%	0%
Hölscher et al. 2009[2]	120	61%	9%	25%	5%
Zhang et al. 2007[2]	376	71%	10%	11%	8%
Ohel et al. 1983	143	54%	14%	NA	NA
Björkholm et al. 1981	198	91%	NA	NA	NA
Evans et al. 1980	108	86%	4%	10%	0%
Stenwig et al. 1979	118	78%	18%	4%	0%
[1]number of patients with stage known. [2]includes 5-10% sex cord-stromal tumors other than AGCT. NA = no data available					

In premenopausal patients, AGCT may present with menstrual irregularity, menorrhagia, amenorrhea, and infertility (6, 14, 73, 79). Postmenopausal bleeding and/or endometrial abnormalities in older women together with a unilateral ovarian mass should raise a suspicion of AGCT (14). A palpable mass is present in most patients (5, 6). In ultrasonography, the appearance of AGCT varies from cystic to solid, although the most common presentation is a solid and cystic mass with occasional hemorrhagic fluid (5, 83, 84) (Figure 2). In preoperative computerized tomography, lymphadenopathy is rarely found in AGCT (83). There are contradictory reports on the fluorodeoxyglucose (FDG) avidity of AGCTs, but mostly it is considered to be low, and thus, F-18-FDG-positron emission (computerized) tomography (FDG-PET/CT) is not recommended in AGCT diagnostics (85-87).

Serum marker inhibin B is established in the diagnosis and follow-up of AGCT, whereas CA12-5 and HE-4, used in epithelial ovarian cancer, are of little value in this disease (18, 83, 88). Anti-Müllerian hormone (AMH), especially in combination with inhibin B, is also a valid serum marker for AGCT, especially in postmenopausal patients (14, 88, 89). However, there are no established cut-off values for inhibin B or AMH in premenopausal patients (14). Even though estradiol (E2) is secreted by the majority of AGCTs, its use as a serum marker suffers from lack of reliable methods, especially among postmenopausal women (18, 90). In general, the currently available E2 immunoassays are sufficiently reliable only in healthy, premenopausal women, and mass spectrometry methods require further standardizing. There is also a need for the development of age- and gender specific reference intervals (90).

AGCTs may have histomorphological patterns similar to many unrelated tumors (14). With the relative rarity of AGCTs, this makes the histological diagnosis challenging, which is demonstrated in studies including re-evaluation of original histological slides. In older series, a 40% false-positive diagnosis rate has been recorded (9). In addition to expert morphologic assessment and immunohistochemical markers such as α-inhibin and calretinin, the highly specific *FOXL2* mutation status is now recommended in the differential diagnosis of AGCTs (14, 91).

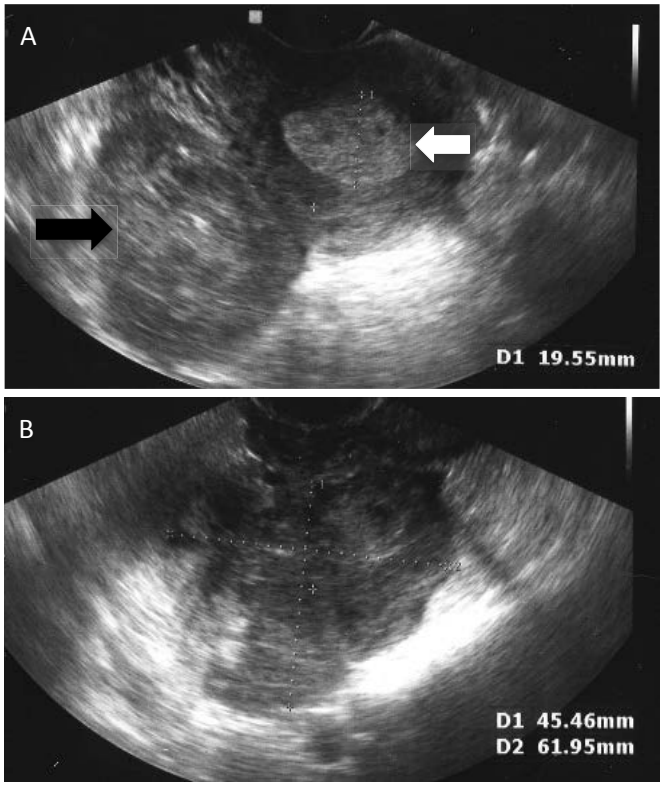

Figure 2. *Ultrasound images of AGCT. A. A largely solid unilateral mass is present in the ovary (black arrow), and a thickened (19.6 mm) endometrium is seen in the uterus (white arrow). B. A mainly solid unilateral mass in the ovary measuring 62 x 46 mm.*

2.2.2 Treatment and follow-up

Despite recent advances in understanding the molecular pathogenesis of AGCT, the developments in specific treatment regimens have remained limited (14). Surgery remains the cornerstone of treatment in both primary and recurrent AGCT (14, 92). When AGCT is suspected, a complete removal of the tumor together with hysterectomy, bilateral salpingo-oophorectomy and staging with peritoneal washings, peritoneal biopsies, and infracolic omentectomy is the treatment of choice (14, 92) (Figure 3). A number of studies have shown very low rates of lymph node involvement in primary AGCT, hence routine pelvic and/or para-aortic lymphadenectomy is not recommended in early-stage disease, and the National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO) guidelines state that lymphadenectomy may be omitted in all stages without evidence of nodal abnormality (92-95). It should be noted that the definition of early-stage or low-risk tumors varies from only stage IA to stage IA-IC up to stage II tumors (92, 93, 95). In premenopausal patients with a stage I tumor, a fertility-sparing surgery with complete staging is an option that does not seem to compromise prognosis (76, 95, 96). Nevertheless, it is not determined whether these patients should undergo total hysterectomy with the removal of the remaining adnex after childbearing is completed or by menopause (95).

Adjuvant treatment

Stage IA AGCTs have a good prognosis after surgical removal and do not require adjuvant treatment (92). In stage IC tumors, the use of adjuvant therapy is not established, but may be considered (92, 95). Overall, the role and efficacy of adjuvant regimens in AGCTs remain inconclusive, but traditionally the most used chemotherapeutic treatment in an advanced setting is the bleomycin/etoposide/cisplatin (BEP) combination (92). The largest prospective study of chemotherapy in AGCT showed an overall response rate of 40% with BEP, with a median duration of response of 24.4 months (97). Alternative options include paclitaxel/carboplatin, docetaxel, paclitaxel, and paclitaxel/ifosfamide (95). The US-based Gynecologic Oncology Group is currently conducting a randomized trial comparing BEP with paclitaxel/carboplatin in the treatment of chemo-naïve recurrent SCSTs (92).

Historically, the adjuvant therapies have included doxorubicin, 5-fluorouracil, and alkylating agents such as cyclophosphamide, but the results with these regimens have been modest (18). Radiotherapy has been used in the treatment of AGCT, particularly in past decades, but adequate evidence for its benefit is lacking (18). In inoperable settings, palliative localized radiotherapy may be considered (18, 95).

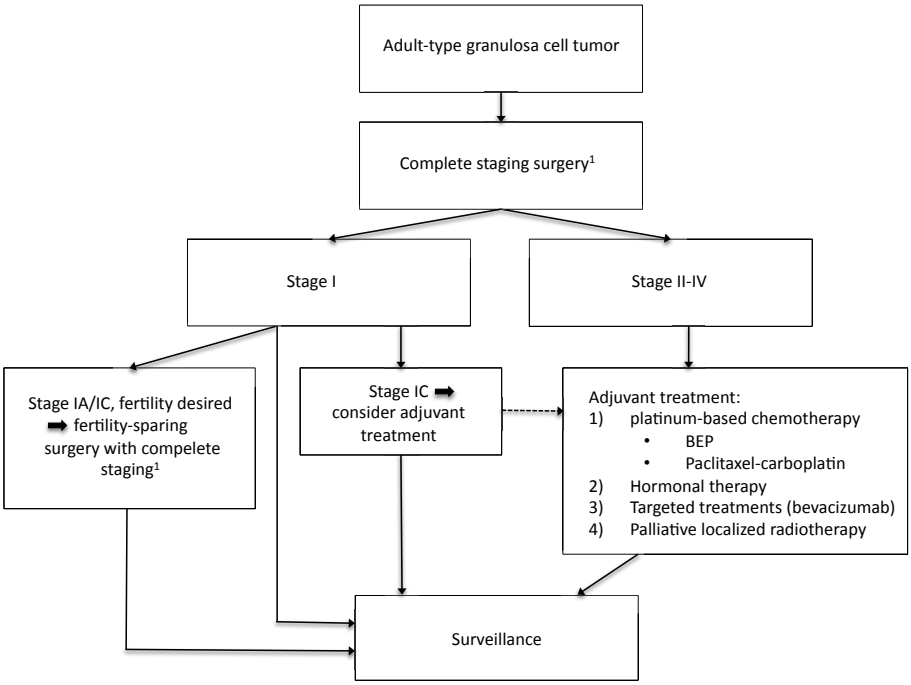

Figure 3. *Treatment of malignant sex cord-stromal tumors. [1]Complete staging = omentectomy, peritoneal cytology (aspiration of ascites or peritoneal lavage), and peritoneal biopsies. Modified from the National Comprehensive Cancer Network (NCCN) 2016 Guidelines (95) .*

Hormonal and targeted treatments have also been investigated in the treatment of advanced or recurrent AGCTs, showing varying efficacy (98). The benefit of these therapies is the low toxicity compared with chemotherapy, leading to better tolerance and less side effects (99). The options include progestins, gonadotropin-releasing hormone (GnRH) agonists and antagonists, selective estrogen receptor modulators (SERMs), and aromatase inhibitors. Even though good response rates have been shown, studies on hormonal treatments suffer from poor quality and lack of prospective data (98, 100). Moreover, hormonal treatments are typically administered after multiple preceding treatments (98). Antiangiogenic agent bevacizumab is a targeted therapy with a potential benefit in the treatment of advanced and recurrent AGCTs, as concluded in the rather recently published phase 2 trial of the Gynecologic Oncology Group (14, 101).

Follow-up

The standard follow-up of AGCT patients typically includes regular gynecologic examinations with pelvic/transvaginal ultrasound and serum tumor markers. As in preoperative evaluation, the most sensitive and specific serum markers are inhibin B and AMH, which are also elevated in relapsed settings and correlate with disease burden (14). The current ESMO recommendation for the duration of routine clinical follow-up is five years, at three-month intervals for the first two years, followed by six-month intervals until the end of follow-up or progression (92). The NCCN recommends physical exam with tumor markers every two to four months for the first two years, and a computerized tomography (CT) scan with tumor markers in case of suspected recurrence. As in ESMO guidelines, the NCCN recommends further follow-up visits every six months, but extends the duration of follow-up beyond five years, although the exact duration is not specified (95). In Finland, the national treatment guidelines for gynecological cancers are based on recommendations by FIN-GOG (Finnish Society of Gynecologic Oncology). In Helsinki University Hospital (HUH), AGCT patients routinely undergo hospital-based follow-up visits for three to five years at four- to six-month intervals; the follow-up includes gynecologic examination, pelvic ultrasound, serum tumor markers, and a CT scan in case of suspected recurrence.

2.2.3 Recurrence and survival

AGCTs are known for their relatively good prognosis and tendency for late relapse (5, 6). In recent studies, the rate of tumor recurrence varies widely from 5% to 64%, and the median time to relapse is between 3.6 and 12 years (77, 102-104) (Table 4). The longest reported intervals to AGCT recurrence are 37 and 40 years after primary diagnosis (105, 106). Due to its typically indolent progression, survival rates in AGCT clearly exceed those of epithelial ovarian cancer (6). In more recent and larger studies including all tumor stages, the 5-year survival rates vary between 93% and 97%, and 10-year survival rates between 87% and 94% (Table 4). The comparison of survival rates in earlier studies suffers from somewhat inconsistent data, where also crude survival rates are presented, i.e. deaths of causes other than GCT are included (11, 107, 108). It is also noteworthy that some series include relatively short follow-up periods, which strongly affects the reliable assessment of recurrence and survival in a slowly proceeding cancer such as AGCT (79, 107, 109, 110). Furthermore, most of these studies lack histological review and/or molecular validation of diagnosis (11, 79, 96, 103, 104, 107, 110).

Typical sites for AGCT recurrence include the pelvis and abdominal cavity, but it may also be seen in the liver, retroperitoneal space, pelvic or para-aortic lymph nodes, bone, and lung (77, 79, 107, 111-113). Multiple-site recurrences have been observed in up to 50% of patients with relapse (107). In case of suspected recurrence, a CT scan with serum

tumor markers is recommended in order to assess the presence of extra-pelvic or multiple-site relapse (95). In local recurrences, surgical debulking is the treatment of choice, but in case of residual or advanced disease, adjuvant treatment is necessary. The alternative treatment modalities are similar to those of advanced disease in a primary setting.

Despite overall favorable prognosis of AGCT, in some series over 70% of patients with recurrent tumors succumb to their disease, emphasizing the value of prognostic tools in identifying patients at increased risk for relapse (74).

Table 4. *Recurrence and survival rates of AGCT in previous studies (series with >100 patients).*

Study	N	Study period	Median follow-up (months)	Relapse rate	Median time to relapse (months)	5-year survival	10-year survival
Karalok et al. 2016	158	1988-2013	97	12.5%	43.5	96.2%	88.2%
Wilson et al. 2015[1]	160	1955-2012	84	32%	144	99.2%	96.4%
Lauszus et al. 2014	163	1962-2003	180	27.6%	NA	NA	NA
Ud Din et al. 2014	156	1992-2012	72[2]	5.1%	NA	NA	NA
Van Meurs et al. 2014	127	1962-2013	131	64%	78	NA	NA
Ertas et al. 2014	108	1991-2010	66[2]	16.6%	61	93.3%[3]	87.0%[3]
Suri et al. 2013	104	1995-2010	31	23.1%	NA	NA	NA
Sun et al. 2012	176	1984-2010	61	21.0%	57.6	96.5%	94.1%
Lee et al. 2011	102	1995-2007	54.7	9.8%	48	NA	NA
Zhang et al. 2007[4]	376	1992-2001	NA	NA	NA	88%	79%
Ohel et al. 1983	172	1960-1975	NA	NA	NA	55%[3]	NA
Björkholm et al. 1980	263	1923-1972	NA	NA	NA	85%	75%
Evans et al. 1980	118	1910-1972	NA	18.6%	72	NA	NA
Stenwig et al. 1979	118	1932-1970	NA	21.2%	106.8[2]	82.3%[3]	NA

[1]only stage Ic tumors. [2]mean value. [3]overall survival. [4] includes 10% sex cord-stromal tumors other than AGCT. NA= no data available

2.3 Prognostic factors

Clinical prognostic factors for AGCT have been proposed in several studies, where endpoints or outcomes include tumor relapse and disease-specific as well as overall survival. However, these studies show varying quality as, in addition to short follow-up periods and lack of histology review, they may include juvenile tumors (11, 74) or suffer from relatively small patient series (114-116). Tumor stage is the most consistent factor for both AGCT relapse and survival in these analyses, along with the presence of residual tumor (11, 77, 79, 103, 109, 110, 114, 116, 117). The significance of other clinical factors is contradictory. The results for main prognostic factors in larger studies are summarized in Tables 5 and 6 and discussed in more detail below.

Table 5. *Clinical patient-related prognostic factors for tumor recurrence and AGCT-specific or overall survival in univariate analyses (series with >100 patients). Independent prognostic factors in multivariate analysis are bolded.*

Study/Endpoint	N	Advanced age	Parity	Postmenopausal status	BMI
Recurrence					
Karalok et al. 2016	158	Yes	-	Yes	-
Wilson et al. 2015[1]	160	No	-	-	-
Ud Din et al. 2014	156	-	-	-	-
Van Meurs et al. 2014	127	No	-	-	**Yes**
Ertas et al. 2014	108	No	-	No	-
Mangili et al. 2013	108	-	-	-	-
Suri et al. 2013	104	No	-	-	No
Sun et al. 2012	176	No	No	No	No
Lee et al. 2011	102	No	-	No	-
Disease-specific survival					
Björkholm et al. 1981	198	No	No	No	-
Evans et al. 1980	118	No	-	No	-
Karalok et al. 2016	158	**Yes**	-	Yes	-
Overall survival					
Ohel et al. 1983	172	Yes	-	No	-
Stenwig et al. 1979	118	Yes	-	-	-

[1]Only stage I tumors included. BMI = body mass index

Table 6. *Clinical tumor- and treatment-related prognostic factors for tumor recurrence and AGCT-specific or overall survival in univariate analyses (series with >100 patients). Independent prognostic factors in multivariate analysis are bolded.*

Study/Endpoint	Tumor size	Tumor rupture	Stage	Residual tumor	Lymph-adenectomy	Adjuvant CT
Recurrence						
Karalok et al .2016	-	Yes	**Yes**	-	No	No
Wilson et al. 2015[1]	No	Yes	Yes[2]	-	-	-
Ud Din et al. 2014	Yes	-	-	-	-	-
Van Meurs et al. 2014	**Yes**	-	**Yes**	Yes	-	No
Ertas et al. 2014	No	No	**Yes**	**Yes**	No	Yes
Mangili et al. 2013	-	-	**-**	Yes	-	Yes
Suri et al. 2013	No	No	-	-	-	No
Sun et al. 2012	**Yes**	-	Yes	**Yes**	No	Yes
Lee et al. 2011	No	No	**Yes**	Yes	-	No
Evans et al. 1980	-	-	Yes	-	-	-
Disease-specific survival						
Karalok et al. 2016	-	No	**Yes**	-	No	No
Björkholm et al. 1981	Yes[1]	Yes[1]	Yes	-	-	-
Evans et al. 1980	-	-	Yes	-	-	-
Overall survival						
Ohel et al. 1983	-	-	Yes	-	-	-
Stenwig et al. 1979	Yes	-	Yes	-	-	-
CT=chemotherapy. [1]only stage I tumors. [2]stage Ia vs Ic						

Age or menopausal status

Patient age and menopausal status have been evaluated as prognostic factors in a number of retrospective hospital-based studies, where menopausal status is typically assessed according to clinical symptoms at the time of diagnosis. Most of these studies report no prognostic value of age or menopausal status at primary diagnosis (77, 79, 103, 107, 109, 117, 118). However, some studies propose advanced age or postmenopausal status as risk factors (76, 96, 104, 119). Furthermore, the reported age categories vary among studies between +/- 40, +/- 50, and +/- 60 years. Only two larger studies suggest that younger and premenopausal patients are at increased risk for relapse, but either the findings are merely marginally significant (110), or significant differences are present in overall survival only when comparing the age groups of 41-50 years and 51-60 years (11). Three recent studies reported advanced age (> 50 years) as an independent prognostic factor for disease-related survival in multivariate analysis (96, 104, 119).

Parity

Only a few studies have assessed parity or reproductive status in AGCT prognosis, but the findings consistently show that reproductive factors do not seem to affect outcome in terms of recurrence or disease-specific survival (74, 79, 118). Only the study by Björkholm et al. (1981) specifies the reproductive categories analyzed (parous, nonparous, or no information) (118).

Symptoms

One study reported a better prognosis for patients presenting with abnormal vaginal bleeding rather than abdominal pain (11). A more recent report evaluated disease presentation with or without symptoms and concluded that there was no statistical difference regarding tumor recurrence (103).

Tumor size and tumor rupture

Larger tumor size has been associated with poorer outcome also in multivariate analysis (79, 103, 117). However, this is not a consistent finding in other series (96, 107, 109, 110, 114). Tumor size has been analyzed both as a continuous variable (103) and as dichotomized values of 5 to 10 cm (96, 109, 110). Two studies used ROC (receiver operating characteristic) curve analysis to estimate the optimal cut-off values for tumor size that predicted recurrence, which were 12.0 and 13.5 cm (79, 117). Ud Din et al. (2014) concluded that tumor size is associated with higher risk of recurrence, but it is unclear which statistical methods were used to verify this finding (102).

Preoperative or perioperative tumor rupture has been proposed to influence particularly the risk for tumor recurrence (77, 104, 118), but there are also contradictory reports on this characteristic (107, 109, 110, 117). Karalok et al. (2016) described the prognostic value of tumor ruptures in terms of recurrence irrespective of whether they were spontaneous or iatrogenic, but in this analysis tumor rupture did not predict disease-specific survival (104). However, in an older Swedish study, the presence of tumor rupture in stage I disease was associated with disease-related mortality (118).

Stage

Tumor stage is the only clinical prognostic factor that is reported positive in virtually all studies and also in multivariate analyses (11, 74, 76, 79, 96, 103, 104, 107, 108, 110, 118, 120). Stage is associated with both disease-related survival (11, 76, 96, 107, 114, 118-120) and tumor recurrence (76, 103, 104, 107, 110). The reported 5-year survival rates in stage I disease range from 75% to 95%, in contrast to 55% to 75% in stage II and 22% to 50%

in stage III/IV tumors (6). For patients with stage I disease, the relapse rate seems to be higher in stage IC, i.e. in case of tumor rupture or tumor infiltrating the ovarian surface (77).

Histology

Histological prognostic factors include mitotic index (MI), poor differentiation of the tumor, and nuclear atypia (104, 118). Several studies have found an association between higher MI and poorer AGCT prognosis (103, 108, 114, 116, 118), and between nuclear atypia and increased mortality (108, 116, 118). Björkholm et al. (1981) reported worse survival in tumors with a high number of mitoses, but concluded that the number of mitotic figures correlated with advanced stage, and in stage I disease there was no difference in survival between tumors with high and low number of mitoses (118). Leuverink et al. (2008) found no correlation with proliferation-associated histological indices such as mitotic activity or Ki-67 index and clinical outcome (121). In a recent study, Karalok et al. (2016) found an association in univariate analysis of poorly differentiated tumors with relapse, but not with disease-related mortality (104). More specific suggestions for histological prognostic markers include adhesion molecules E-cadherin and β-catenin, human epidermal growth receptor 2 (HER2), and transcription factor GATA4, but their clinical applicability is unresolved (122, 123).

Presence of residual tumor

In four rather recent studies, the presence of residual tumor after primary treatment was associated with AGCT recurrence in multivariate analysis, highlighting the need for complete cytoreduction at the primary setting (79, 107, 115, 117). Several univariate analyses have shown similar results (76, 103, 110). Hölscher et al. (2009) presented a decreased overall and relative survival in patients with residual tumor (120). However, a recent study found no association between residual disease at primary surgery and disease-related survival (119).

Treatment

The effect of treatment on AGCT prognosis has been evaluated in terms of complete or fertility-sparing surgery, presence of surgical staging, performance of lymphadenectomy, and need for adjuvant treatment. In a large study on stage I AGCTs, no significant increase in relapse rate was found in premenopausal patients undergoing fertility-sparing surgery (77), and Zhang et al. (2007) presented similar findings in an extensive cohort of SCSTs (96). An older study concluded that the risk of recurrence was higher in patients without total abdominal hysterectomy and bilateral salpingo-oophorectomy (74). Mangili et al. (2013) found in multivariate analysis that incomplete surgical staging and treatment outside a referral center were prognostic factors for recurrence, but not for disease-specific survival (119).

Several authors have concluded that providing lymphadenectomy or adjuvant treatment has not affected prognosis (103, 104, 109, 110, 117, 119), although these have shown prognostic value in some univariate analyses, most likely related to disease stage (76, 79, 107, 120). A recent study on stage IC AGCTs reported that adjuvant chemotherapy had no predictive value for recurrence, but in this series only nine patients received chemotherapy (124). Two older studies demonstrated that the use of postoperative radiotherapy was not associated with poorer prognosis (11, 74).

Other clinical characteristics

Suri et al. (2013) evaluated demographic characteristics, such as race, body mass index (BMI), and presence of diabetes, in relation to progression-free survival in AGCT (109). This study found an independent association between diabetes and tumor relapse, whereas race or BMI over 30 kg/m2 did not significantly affect the outcome. Two other studies have assessed BMI as a prognostic factor for AGCT recurrence, with somewhat differing results (79, 103). In the study by Sun et al. (2012) it is unclear whether BMI was analyzed as a categorized or continuous variable, but the study concluded that BMI was not associated with disease recurrence (79). Van Meurs et al. (2014) used continuous values for BMI in their study and found a higher BMI to decrease the recurrence-free survival of patients with AGCT, also in multivariate analysis (103).

2.4 Conclusions of the literature review

Although the molecular pathogenesis of AGCT is slowly unravelling with the identification of *FOXL2* mutation status, the specific etiology of these tumors remains unclear. More evidence is needed to clarify the role of the environmental, occupational, and reproductive risk factors typically related to epithelial ovarian cancer in AGCT. There is a lack of modern, population-based studies evaluating the incidence of second primary cancers in patients with AGCT, even though the risk for concomitant endometrial pathology is well documented. Tumor stage is an established clinical prognostic factor for AGCT, but the management, follow-up, and other prognostic factors of this rare disease need to be assessed and analyzed based on long-term, high-quality studies with an accurate selection of sufficient patient series.

3 Aims of the study

This study was undertaken to evaluate the epidemiological background and occurrence of second primary malignancies among patients with AGCT, and to investigate the clinical characteristics and prognostic factors related to AGCT-specific survival and tumor recurrence.

Specific aims of the study were to analyze the following:

1) the incidence and occupational risk factors of AGCTs in an international cohort (I)

2) the incidence of other, particularly estrogen-related malignancies among Finnish AGCT patients, both before and after diagnosis (II)

3) the clinical characteristics, survival, and prognosis of AGCT in relation to development of diagnostics and treatment over the past decades (III)

4) the clinical picture of AGCT relapse and the optimal follow-up strategy for these patients (IV)

4 Materials and methods

These studies were conducted between 2011 and 2017 at the Helsinki University Hospital (HUH). The Ethics Committee of Helsinki University Hospital (197/E9/06 and 210/13/03/03/2016) and the National Supervisory Authority of Welfare and Health in Finland (THL/1469/5.05.00/2012) approved the study protocol. Study I was carried out in collaboration with the Finnish, Icelandic, Norwegian, and Swedish Cancer Registries, and Study II in collaboration with the Finnish Cancer Registry. Studies III and IV examined hospital-based patient series from HUH.

4.1 Study material

The study materials are summarized in Table 7.

Table 7. *Study material in Studies I-IV. The numbers in parentheses represent the final patient cohort after histological and/or molecular validation.*

Study	N	Study period	Source of material
I	2195	1953-2012	National Cancer Registries of Finland, Iceland, Norway, and Sweden
	776	1961-2005	Nordic Occupational Cancer Study (NOCCA)
II	986	1968-2013	Finnish Cancer Registry
III	240 (187)	1956-2012	Patient registry of the Department of Obstetrics and Gynecology and the pathology registry of Department of Pathology, Helsinki University Hospital (HUH)
IV	240 (164)	1956-2014	Patient registry of the Department of Obstetrics and Gynecology and the pathology registry of Department of Pathology, Helsinki University Hospital (HUH)

4.1.1 Study I

AGCT incidence

The Nordic countries have a long history of nationwide, population-based cancer registration systems (40). Utilizing these data, the aim in Study I was to analyze the long-term multi-national incidence of AGCT. The cancer registry data on AGCTs were

extracted from the national registries and available as follows: in Finland from 1968, in Iceland from 1958, in Norway from 1953, and in Sweden from 1993. Starting from these years, the incidence rates were calculated for five-year periods until 2012. Additionally, in the HUH region in Finland, the cases were cross-checked for accuracy with the hospital pathology department.

Occupational analysis

The Nordic Occupational Cancer Study (NOCCA) presents cancer incidence data by occupational category for the Nordic populations based on population censuses in 1960, 1970, 1980/1981, and/or 1990 (astra.cancer.fi/NOCCA). From the census records, the occupational information has been classified into 54 broad categories in the NOCCA database (Table 8).

The occupational variation in the risk of developing AGCT was evaluated using this database. The cohort included a total of 6.4 million women: 1.7 million in Finland, 60,000 in Iceland, 1.3 million in Norway, and 3.4 million in Sweden. All women aged 30-64 years were followed until emigration, death, or to December 31 of the following years: in Finland 2005, in Iceland 2004, in Norway 2003, and in Sweden 2005.

Table 8. *Occupational categories in the NOCCA-database*

1. Technical workers	19. Forestry workers	37. Chemical process workers
2. Laboratory assistants	20. Miners	38. Food workers
3. Physicians	21. Seamen	39. Beverage workers
4. Dentists	22. Transport workers	40. Tobacco workers
5. Nurses	23. Drivers	41. Glass makers etc
6. Assistant nurses	24. Postal workers	42. Packers, loaders
7. Other medical workers	25. Textile workers	43. Engine operators
8. Teachers	26. Shoe and leather w.	44. Public safety workers
9. Religious and juridical w.	27. Smelters	45. Cooks and stewards
10. Artistic workers	28. Mechanics	46. Domestic assistants
11. Journalists	29. Plumbers	47. Waiters
12. Administrators	30. Welders	48. Building caretakers
13. Clerical workers	31. Electrical workers	49. Chimney sweeps
14. Sales agents	32. Wood workers	50. Hairdressers
15. Shop managers, assistants	33. Painters	51. Launderers
16. Farmers	34. Other construction w.	52. Military personnel
17. Gardeners	35. Bricklayers	53. Other workers
18. Fishermen	36. Printers	54. Economically inactive
w. = workers		

4.1.2 Study II

The Finnish Cancer Registry (FCR) maintains a nationwide database on all cancer cases in Finland (www.cancer.fi). Physicians, hospitals, and pathology and hematology laboratories are required to notify the FCR of all incident cancer cases, resulting in a virtually complete registration of cancer cases (125). FCR information can be linked to various information sources via personal identity codes. The Population Register Center provides information on vital status and emigration. In addition, the files are annually matched with cause of death information from Statistics Finland.

For the analysis of second primary malignancies among AGCT patients, all cases of primary AGCT in Finland in 1968-2013 were identified from the FCR, applying the ICD-O-3 topography code C56.9 with morphology and behavior codes M8620/1, 8620/3, 8621/1, and 8621/3. Second primary tumors were grouped in 18 broad categories based on cancer site (Table 9). Subanalyses for second cancers were performed in subcategories of breast cancer (local and invasive), female genitalia (endometrial cancer), lymphatic and hematopoietic tissue (leukemia), and urinary tract (bladder cancer).

Table 9. *Primary tumor categories in the analysis of second primary tumors (Study II).*

C00-14 Oropharynx
C15-C26 Gastrointestinal tract
C30-39 Respiratory tract
C40-41 Bone
C43 Skin, melanoma
C44 Skin, non-melanoma
C45 Mesothelioma
C47 Autonomic nervous system
C49 Connective tissue
C50 Breast
C51-58 Female genitalia
C64-68 Urinary tract
C69 Eye
C70-72 Brain, central nervous system
C73 Thyroid
C75 Other endocrine organs
C76-80 Other or undefined
C81-96 Lymphoid and hematopoietic tissue

4.1.3 Studies III and IV

The Department of Obstetrics and Gynecology (also Women's Hospital) in HUH is a tertiary referral center responsible for the gynecologic cancer treatment of 1.9 million inhabitants in Southern Finland. As a part of the largest hospital district in Finland, the unit provides complex cancer surgery and adjuvant therapies including chemotherapy and radiotherapy.

The aim in Studies III and IV was to thoroughly evaluate the clinical characteristics, recurrence pattern, survival, and prognostic factors for AGCT in one of the largest single-institute patient cohorts to date. These studies included all AGCT patients treated in HUH from 1956 to 2012 (Study III) and to 2014 (Study IV). In order to histologically and molecularly validate the patient series, the original histological slides were reevaluated by an expert gynecologic pathologist (Study III), and the *FOXL2* mutation status was defined for the whole cohort (Study IV) (Figures 4 and 5). The details of the mutation analysis are described in the study by McConechy et al. (2016), where the AGCT data from three European centers (n=336) was validated for the *FOXL2* mutation in Vancouver, Canada, using allelic discrimination assays (126). The HUH patient cohort comprised the vast majority (67%) of these cases.

Patients who were initially misdiagnosed, lacked a histological sample, or had follow-up for less than one year (Study IV) were excluded from analyses. Three cases with a missing histological sample were included in Study III after a thorough evaluation of original pathology and clinical reports. Following histological/molecular validation, the clinical data for the remaining study population were retrospectively collected from patient files. These included information on age, BMI, parity, menopausal status, use of hormonal therapies, other primary tumors, initial symptoms, tumor size, stage, endometrial pathology, and treatment and follow-up regarding both primary and recurrent tumors. Based on operative reports, tumor stage was redefined retrospectively by the author and a gynecologic oncologist, according to the FIGO 2009 criteria (127). Survival data were obtained from the Finnish Population Register Center and cause of death from Statistics Finland.

In Study III, the focus was on clinical factors affecting survival in relation to major developments in diagnostics and treatment strategies over time. For this purpose, the data were grouped into two categories (1956-1983 and 1984-2012) based on the introduction of platinum-based chemotherapy and the increased use of modern imaging techniques such as vaginal ultrasound and CT scans in our department. In Study IV, we evaluated the prognosis in terms of tumor relapse in patients who were considered disease-free after primary treatment. For analysis, recurrence sites were grouped according to anatomical regions.

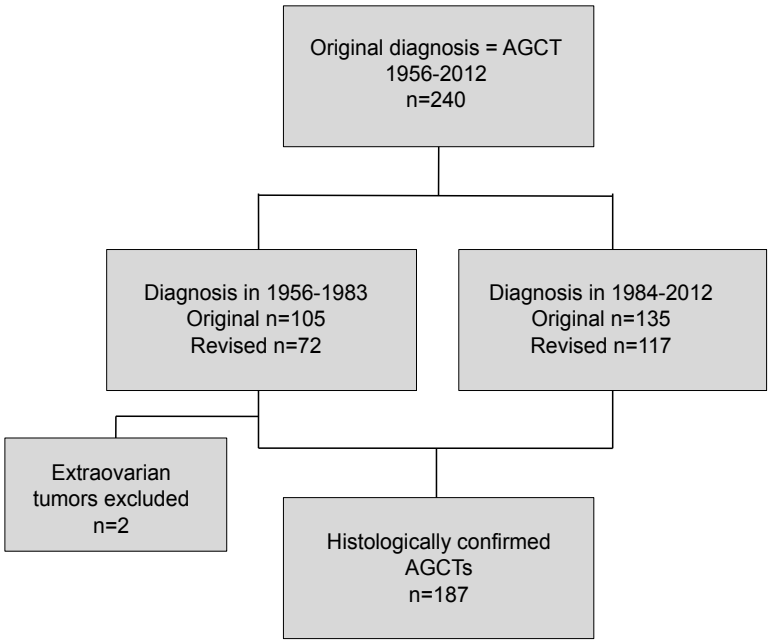

Figure 4. *The histologically revised Helsinki University Hospital AGCT patient cohort in Study III*

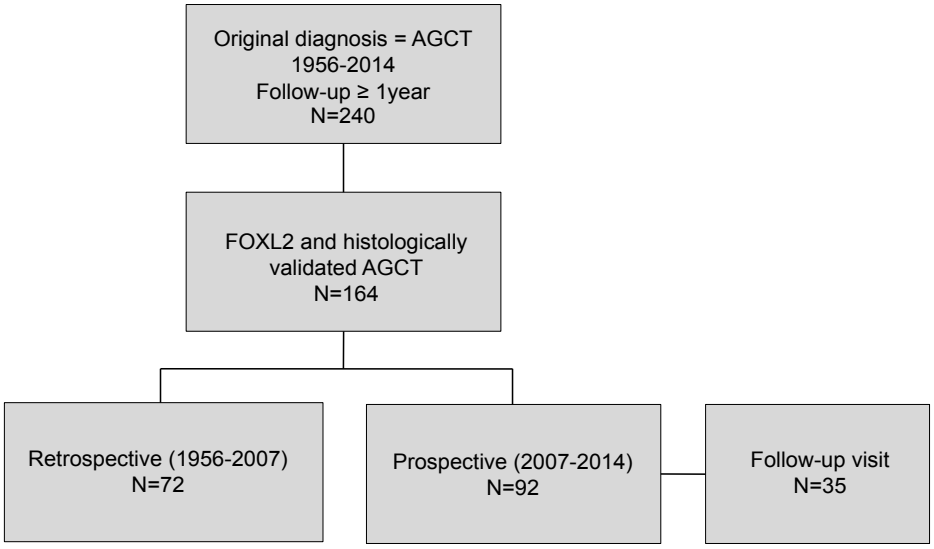

Figure 5. *The histologically and molecularly revised Helsinki University Hospital AGCT patient cohort in Study IV.*

Additionally, from 2007 onwards, living patients have been invited with their informed consent to a prospective follow-up study with a structured questionnaire on reproductive and hormonal factors and family cancer, supplemented with a clinical follow-up visit with gynecologic examination, pelvic ultrasound, and serum tumor markers every five years after the routine surveillance has concluded. By 2014, a total of 35 women from this series had undergone prolonged surveillance for the prospective study, 19 of whom had one and 16 two clinical visits every five years (Figure 5).

4.2 Methods

4.2.1 Studies I and II

Study I

The incidence rates for Finland, Iceland, Norway, and Sweden were calculated for age groups of 20 years and older, and by adjusting the total incidence rate to the World Standard Population. The World Standard Population reflects the age structure of the world's population, allowing comparison of incidence rates by taking into account the relative differences in age distributions.

Due to small number of cases in Iceland, a 15-year floating average in the total incidence rate was used. The differences between the incidence rates were statistically analyzed using Quasi-Poisson regression models. Quasi-Poisson models were used since the assumption that the variance is equal to the mean was not met, which is required by the Poisson model. Quasi-Poisson regression models correct for the overdispersion of the data.

In the occupational analysis, the ratio of observed to expected AGCTs in the different occupational categories was defined as a standardized incidence ratio (SIR). The cancer incidence rates for the national study populations were used as a reference. A 95% confidence interval (CI) was defined for each SIR, assuming a Poisson distribution for the observed number of cases.

Study II

All AGCT patients were identified and followed up for second primary cancer from the date of primary cancer diagnosis to date of death, migration, or end of follow-up on December 31st 2013. First, the number of SPMs among AGCT patients was compared with the expected number of cases calculated by multiplying the person-years with the corresponding population rate, and SIRs and 95% confidence intervals were defined. A subanalysis was then performed for breast cancer after AGCT as well as for AGCT after breast and uterine cancer, where the SIRs were also stratified for time from first cancer diagnosis (0-4 years, 5-14 years, and more than 15 years), age at primary diagnosis (below 50 years or 50 years and older), and breast cancer invasion (local versus invasive). To identify concomitant cancers as well as strong surveillance bias, all second primary cancers and those occurring within six months of primary diagnosis were analyzed separately.

4.2.2 Studies III and IV

The comprehensive clinical data were assembled into an electronic database using Study IDs for individual patients (File Maker Pro 14.0.4, FileMaker Inc., FileMaker International) (Figure 6). The database allows visually simple and modifiable layouts for large volumes of data, from which preferred variables can be exported as a Microsoft Excel File. The data were imported to a statistical software for specific statistical analyses (JMP Pro 10.0.2, SAS Institute Inc., Cary, NC, USA).

The statistical analyses were conducted using cross-tabulation and parametric and non-parametric testing according to distribution. Continuous variables were analyzed for normal distribution using the Shapiro-Wilk test and compared with either Student's t-test or a Mann-Whitney test. Pearson's Chi-squared test or Fisher's exact test was used for analyzing associations between groups.

Disease-specific survival was defined from the date of primary diagnosis to the date of death from AGCT (Study III), and disease-free survival (Study IV) from the date of primary diagnosis to the first confirmed recurrence or last follow-up. Overall survival included death of any cause. The survival curves were calculated using the Kaplan-Meier method and displayed leaving a minimum of five subjects at risk. The statistical significances were estimated with a log-rank test. Prognostic factors were evaluated with univariate and multivariate analyses using Cox's regression model; statistically significant variables in the univariate tests were included in the multivariate models. A P-value of less than 0.05 was considered significant.

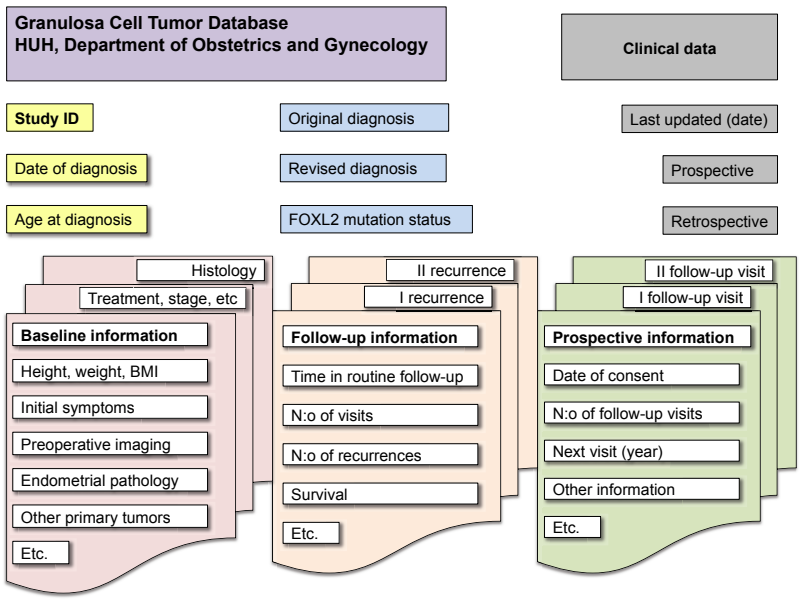

Figure 6. *Schematic illustration of the FileMaker database containing the clinical AGCT data.*

5 Results and discussion

5.1 Incidence and occupational risks of AGCT in Finland, Iceland, Norway, and Sweden (I)

5.1.1 Nordic incidence of AGCT in 1953-2012

The total number of AGCTs in the Nordic study cohort was as follows: 966 in Finland, 685 in Norway, 517 in Sweden, and 27 in Iceland. The incidence remained relatively stable over the study period of 1953-2012, around 0.6-0.8/100,000 (Figure 7B). No increasing or decreasing trend could be seen over time in any of the four countries, but in Finland, the incidence varied more, and was significantly higher in 1993-2012 than in Norway (RR 1.38) or Sweden (RR 1.49). The Finnish incidence was also notably high in 1968-1977.

In specific age categories, the incidence was highest in the age groups of 50-64 years, but remained relatively high even in 65- to 84-year-olds (Figure 8A). In a subanalysis of periods from 1993 to 2002 and from 2003 to 2012, the incidence peak in 55- to 64-year-olds was more evident in the earlier time period (Figure 8B). In 2003-2012, the incidence remained high in the age groups of 55-74 years.

Although a few population-based studies have addressed the incidence of AGCT, many date back several decades and none have combined data from more than one country (9-12). Only one report has evaluated the changes in AGCT incidence over time; it is based on the FCR data for 1965-1994 (12). Björkholm et al. (1980) analyzed the incidence of both granulosa and theca cell tumors in Sweden in 1958-1972 and reported an average crude GCT incidence rate of 0.72/100,000, which is often incorrectly referred to as 1.6/100,000, which was, in fact, the incidence rate for both GCTs and thecomas combined (9). The highest incidence rates (1.4/100,000) have been reported by Lauszus et al. (2014) in Denmark in 1962-2003, but this was not a nationwide, cancer registry-based study (13). The incidence presented in our study is in line with earlier rates. However, the comparison of incidence rates between studies should be done with caution since it is often unclear how the rates have been calculated and adjusted, especially in older analyses.

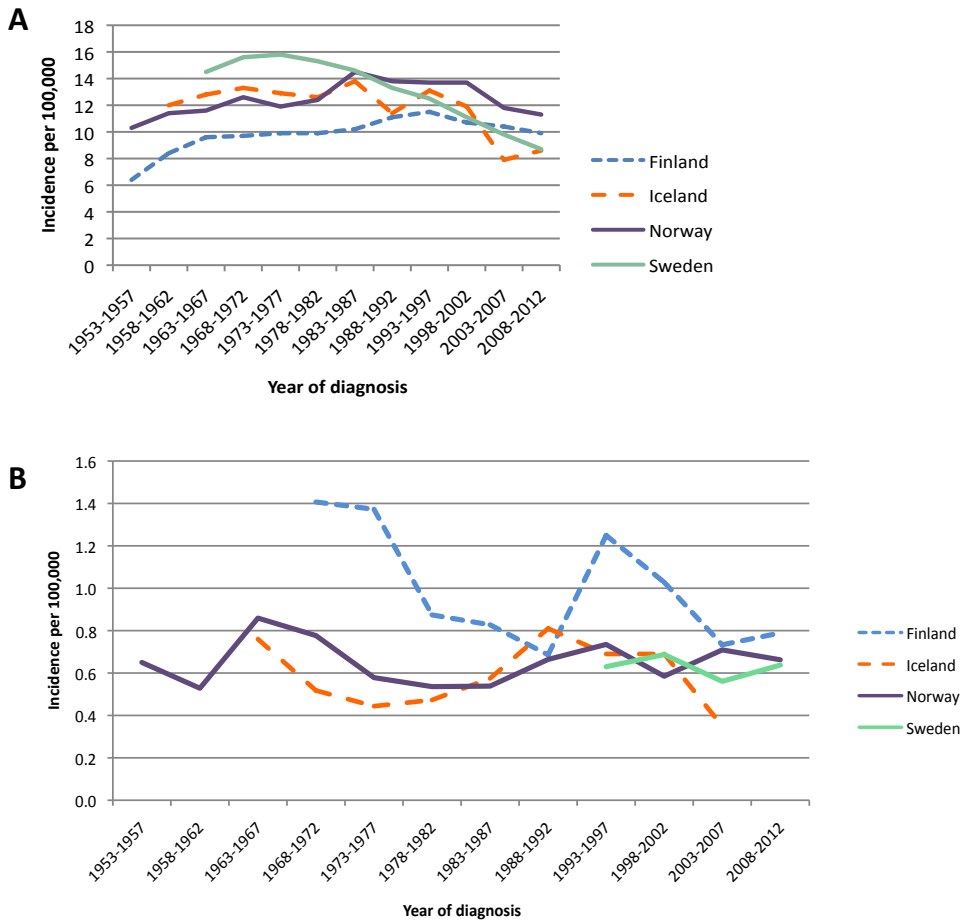

***Figure 7**. Nordic incidence rates of ovarian cancer and AGCT. A. Ovarian cancer incidence rates in Finland, Iceland, Norway, and Sweden in 1953-2012 in 5-year periods, adjusted for age to the World Standard Population (source: Nordcan database). B. AGCT incidence rates in 1953-2012 in 5-year periods, adjusted for age to the World Standard Population, truncated to ages 20 +. The rates for Iceland are presented as floating 15-year averages.*

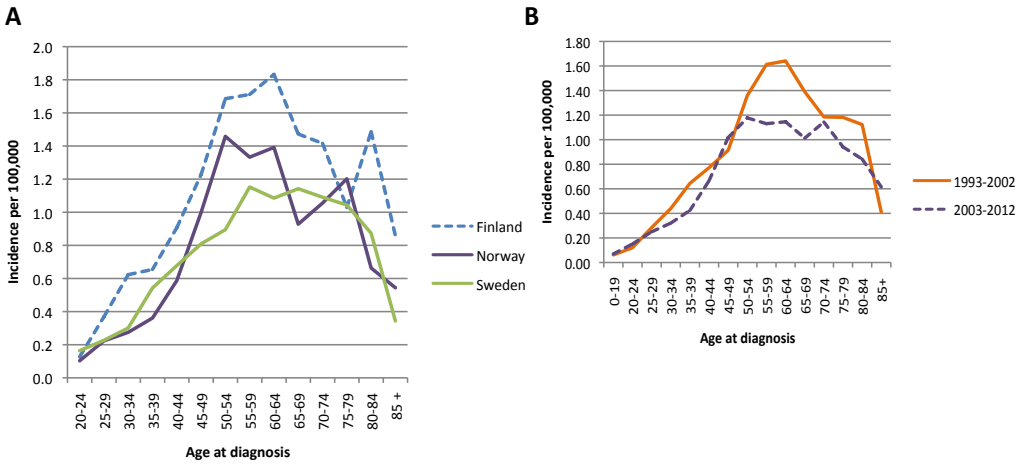

***Figure 8**. A. Age-specific incidence of AGCT in Finland, Norway, and Sweden in 1993-2012, Iceland excluded due to the small number of cases. B. Age-specific incidence rates of AGCT in Finland, Iceland, Norway, and Sweden in 1993-2002 and 2003-2012.*

In our series, the several decade-long study period allows also observations about the incidence trends of AGCT. As a background setting, the overall ovarian cancer incidence was quite stable (Finland, Norway) or decreasing (Sweden, Iceland) in 1953-2012 (Figure 7A) (128). Over time, significant changes in risk factors such as reproductive behavior, use of hormonal therapies, obesity, and smoking may occur. Moreover, both the diagnostic accuracy and criteria may evolve, especially in rare diseases. Indeed, notable changes in lifestyle and reproductive factors have taken place during the study period in the Nordic countries; among women, higher education and participation in the labor market has increased, average parity has decreased, use of oral contraceptives and HRT has both increased and decreased, and obesity has become more common (40, 129). Many of these factors are thought to at least partly explain the reported increase in incidence of breast cancer in the past decades (40, 129). Despite these environmental and reproductive changes, the incidence rates of AGCT have remained rather constant in the four Nordic countries included. The variation in the Finnish incidence was more noticeable, with higher rates in 1968-1977 and 1993-2002, and explanations for this remain unclear. An earlier study based on the FCR data suggested that the increased use of oral contraceptives or development in diagnostics might explain the decrease in the AGCT incidence after the 1970s, but the new increase in the 1990s does not support this (12). In all Nordic countries, the use of HRT increased from the 1970s until the late 1990s, and decreased markedly in 1999-2005, but the decrease was less obvious in Finland than in other countries (129). These changes in HRT use do not appear to have affected the incidence of AGCT, although causality between these factors cannot be determined based on these data. As the recent decades are characterized by an increasing life expectancy and

developments in diagnostics, a slight increase in a slow-proceeding cancer, such as AGCT, could be hypothesized. On the other hand, the widespread use of oral contraceptives might have an effect in the opposite direction.

The diagnosis of AGCT is difficult and prone to misclassification, as shown in several studies (9, 108, 126, 130). Furthermore, there has been dispute as to the malignancy of AGCT – this is well demonstrated in the ICD-O-3 morphology codes 8620/1 and 8621/1 for AGCT, which represent "uncertain" behavior, even though all AGCTs should be considered malignant (9, 131). This may have resulted in underreporting of AGCTs to cancer registries or inconsistencies between different countries, particularly in past decades, and limits the reliability of epidemiological analyses (7). Although the histopathologic diagnostic criteria for AGCT has remained rather uniform since the 1960s, the classification of ovarian tumors has evolved, and also sex cord-stromal tumors have undergone changes in organization as the understanding of rare or previously unclassified sex cord-stromal tumors has developed (131, 132). As previously mentioned, the diagnostic accuracy has improved since the introduction of immunohistochemistry and molecular validation. These factors are likely to partly influence the registry accuracy and AGCT incidence over longer time periods.

The incidence peak of AGCT in the menopausal or early postmenopausal period is in line with previous studies and reflects the hypergonadotropic hypogonadal state associated with ovarian cancer in general, although the gonadotropin hypothesis is under debate (68, 133-135). The role of follicle stimulating hormone (FSH) in AGCT pathogenesis is equally unclear (14). Although it is known that granulosa cells express FSH receptors, the few treatment trials using GnRH agonists or antagonists have shown modest results (5, 100, 136). Two case reports have identified an unusual elevation of serum luteinizing hormone (LH) in premenopausal AGCT patients, but the mechanism for this elevation is unknown (137, 138).

The risk for AGCT seems to be relatively high also later in menopause - up to 80 years of age - compared with premenopausal age categories. Relative to epithelial ovarian cancer during the same study period, the incidence peak in AGCT is seen in earlier age categories, and declines more steeply after 80 years of age (128). AGCTs in patients under 30 years of age are very rare, and series containing a large number of young patients should be suspected of including juvenile GCTs.

5.1.2 Occupational analysis

In the NOCCA cohort, a total of 776 AGCTs were observed: 195 in Finland, 7 in Iceland, 341 in Norway, and 233 in Sweden. The highest SIRs were seen in welders, dentists, forestry and transport workers, and printers (Table 10). However, the observed number of cancer cases was small in these categories, and the SIRs were not statistically significant. In a period- and age-specific subanalysis, the SIR was significantly increased among building caretakers in 1991-2005 (1.51, 95% CI 1.01-2.17), among printers aged 30-49 years (SIR 6.40, 95% CI 1.74-16.4), among transport workers and building caretakers aged 50-69 years (SIR 5.55, 95% CI 1.14-16.2), and among packers aged 70 years or over (SIR 3.25, 95% CI 1.06-7.59) (data not shown).

Nurses, waiters, hairdressers, and teachers had lower risks of developing AGCT than the reference population (Table 10). Teachers had a significantly decreased SIR of 0.64 (95% CI 0.38-0.99).

Table 10. *Observed numbers of AGCTs in Finland, Iceland, Norway, and Sweden, and standardized incidence ratios in 1961-2005 in selected occupational categories.*

Occupational category	Obs	SIR	95 % CI
Welders	1	3.78	0.10-21.1
Dentists	3	3.65	0.75-10.7
Forestry workers	1	3.35	0.08-18.7
Transport workers	3	3.15	0.65-9.20
Printers	5	2.11	0.69-4.92
Packers	11	1.40	0.70-2.51
Building caretakers	51	1.30	0.97-1.71
Textile workers	21	1.12	0.70-1.72
Assistant nurses	19	0.95	0.57-1.49
Nurses	12	0.84	0.43-1.46
Waiters	8	0.83	0.36-1.63
Hairdressers	3	0.75	0.15-2.19
Teachers	19	**0.64**	**0.38-0.99**
Obs = observed number of cases. SIR = standardized incidence ratio. CI = confidence interval.			

To our knowledge, we are the first to present data on occupational risk factors for AGCT. In contrast to previous studies in occupational risks for mainly epithelial ovarian cancer, we did not detect an increased risk among nurses or teachers, although the number of cancer cases was rather small in these occupational categories. On the contrary, the risk for AGCT in these occupational categories was decreased in our analysis, particularly among teachers. This supports the assumption that the risk factors for distinct subtypes of ovarian cancer differ from each other. No single occupation demonstrated a clearly and significantly increased risk for AGCT, which indicates that no specific occupational exposure is directly associated with this tumor. The elevated risk among building caretakers, textile workers, printers, and packers may be linked to chemical exposure or less use of health care services, and these occupations typically have increased risks for several types of tumors (40). Of particular interest is the slightly decreased risk among waiters, who have been shown to have significantly increased SIRs of lung and oropharyngeal cancer related to cigarette exposure (40). This is in line with an epidemiological study that found the risk for developing AGCT to be lower in women who smoked (31). However, this association cannot be confirmed based on our data.

It should be recognized that the occupational analyses suffer from low power and may not be sufficient in detecting all relevant associations. Furthermore, it is difficult to discriminate between the effects of potential confounding factors in occupational categories such as parity, BMI, smoking, or use of hormonal therapies. It is known that highly educated women typically share similar reproductive factors, such as relatively low parity and higher age at first childbirth, which is also demonstrated in the higher rates of breast cancer among both teachers and dentists (40). However, these two occupations showed opposite risks for developing AGCT, although the observed number of AGCTs among dentists was very small. Future investigations call for well-designed epidemiological analyses with adequate power, which are able to take into account also potential hormonal and reproductive confounders.

5.2 Second primary malignancies in patients with AGCT (II)

5.2.1 Second primary cancers after AGCT

In 1968-2013, altogether 986 women presented with AGCT in Finland with a total of 122 second primary cancers and a median interval of 19.2 years between cancer diagnoses (range 0.02-45.6 years, all cases included) (Figure 9). Thus, the rate for SPM among AGCT patients was 12.4%, and if also cancers diagnosed within six months of the primary diagnosis were included, the rate was 13.9%. These figures are slightly above the expected rates (SIR 1.09, 95% CI 0.91-1.3 and SIR 1.19, 95% CI 1-1.41, respectively) (Table 11).

Of specific cancer types, the SIRs were significantly increased for cancers of the soft tissue, thyroid, and leukemia following the first primary AGCT (Table 11). There were also more than expected cases of cancers of the oropharynx, breast, urinary organs, skin (non-melanoma), and mesothelioma, albeit not significantly (data not shown). When stratified for follow-up time (less than five years, 10-15 years, and more than 15 years), the SIR was significantly increased for all second primary cancers only after 15 years from the initial primary AGCT diagnosis (SIR 1.40, 95% CI 1.07-1.78).

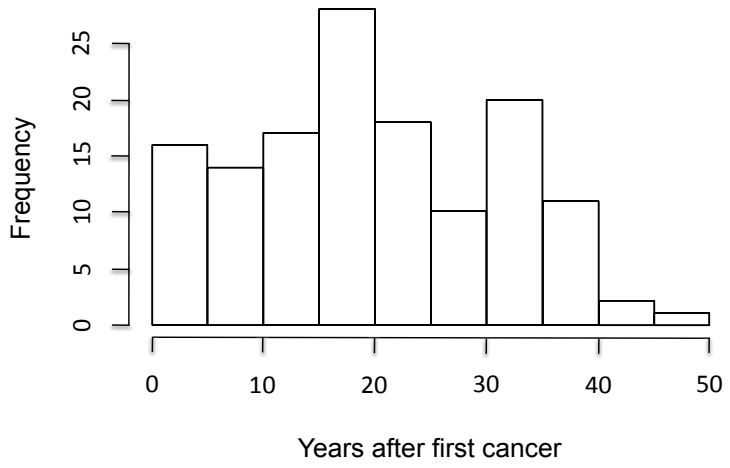

***Figure 9**. Delay for second primary cancer after AGCT, by five-year intervals. The median interval between cancer diagnoses was 19.2 years (range 0.02-45.6 years).*

Table 11. *Risk of subsequent primary malignancies among women with previous AGCT in Finland in 1968-2013 in selected cancer sites.*

Cancer site	Observed	Expected	SIR	95 % CI	p-value
All, diagnosis within 6 months of AGCT included	137	114.7	1.19	1-1.41	0.04
All, diagnosis > 6 months after AGCT	122	111.7	1.09	0.91-1.3	0.33
Soft tissues	3	0.7	4.13	1.33-12.8	0.01
Breast	38	30.2	1.26	0.92-1.73	0.15
Thyroid gland	6	1.8	3.42	1.54-7.62	0.003
Leukemia	6	2.2	2.67	0.98-5.82	0.03
Bladder and urinary tract	3	2.4	1.27	0.26-3.73	0.92
SIR= standardized incidence ratio, CI = confidence interval.					

Obesity, female hormones and reproductive factors have been suggested to play a role in thyroid cancer pathogenesis, but no consistent association between ovarian and thyroid cancer has been shown (139-141). Interestingly, Björkholm et al. (1980) also found the risk for second primary thyroid cancer to be increased, which is the only previous study evaluating the incidence of all second primary malignancies among AGCT patients (9). Both secondary soft tissue cancer and leukemia, on the other hand, are strongly associated with carcinogenic treatment regimens, notably radiotherapy and chemotherapy, respectively (142, 143). However, radiotherapy is extremely seldom used as an adjuvant treatment in AGCT, although it was somewhat more common in the past decades (18). In addition, radiotherapy for ovarian cancer is known to predispose to secondary bladder carcinoma, which was not significantly increased after AGCT in our analyses (Table 11). Thus, shared risk factors are more likely to explain the increased SIR for both soft tissue cancer and thyroid cancer among these women. The increased incidence of leukemia is most likely due to late carcinogenic effects of chemotherapy for AGCT. Adjuvant platinum-based chemotherapy is often used in advanced or recurrent AGCT, and an increased risk for leukemia is a known secondary effect of chemotherapy (60, 143). These findings are also consistent with two population-based studies that reported increased SIRs for colorectal, lung, breast, bladder, and thyroid cancer as well as for leukemia after ovarian cancer of any type (59, 60). In these analyses, the increased risk for secondary malignancy was further associated with older patient age and chemo- and radiotherapy for primary ovarian cancer.

5.2.2 AGCT and breast cancer

The risk for breast cancer after AGCT was increased 1.3-fold in total, and 1.4-fold after at least five years of primary cancer diagnosis, albeit not significantly (Table 12). Subsequent breast cancers presented slightly more often in women who were 50 years or older at the time of AGCT diagnosis. It is noteworthy that the increased risk seemed to be confined to only localized breast cancers (SIR 1.36), as the SIR in non-localized cancer was 0.83.

Women with a first primary breast cancer had a significantly increased SIR of 1.59 for developing a second primary AGCT (95% CI 1.04-2.29) (Table 12). The risk was over 2-fold in breast cancer patients less than 50 years of age at the primary diagnosis, and in patients with more than 15 years from breast cancer diagnosis.

These results indicate that there is an increased risk for these two cancers among the same women, especially in the case of a first primary breast cancer. This finding is in line with a few earlier studies, which have demonstrated more than expected cases of breast cancer among AGCT patients, and a breast cancer rate of 5-10% among this group (11, 71, 75). In our study, the total rate was 6.9%, including breast cancers before and after AGCT. Hammer et al. (2013) reported an odds ratio of 3.3 among 163 women with AGCT, including breast cancer both prior to and after AGCT, whereas Ohel et al. (1983) and Meisel et al. (2015) found the risk to be increased in women with a first primary breast cancer in studies with 172 and 118 AGCTs, respectively (11, 75). Evans et al. (1980) and Björkholm et al. (1980) studied patients with granulosa and theca cell tumors, reporting incidences of breast cancer among these women to be 2-5.5% (9, 74).

The finding that the risk for subsequent breast cancer after AGCT is confined to localized cancer only supports the role of surveillance bias, i.e. the increased intensity of clinical follow-up and examination in patients with a prior cancer diagnosis. A tendency towards long latency between the two cancers is seen, regardless of which tumor was primarily diagnosed, although the SIR for AGCT after breast cancer is elevated already in the shortest follow-up category (Table 12). Mellemkjaer et al. (2006) found an overall increased risk for second cancer after breast cancer in a cohort of over 500,000 women with primary breast cancer, which was explained by the effects of breast cancer treatment, shared genetic and environmental risk factors, and surveillance bias (144). Furthermore, their study observed elevated risks for ovarian cancer after breast cancer as well as for breast cancer after a first primary ovarian cancer, which is most likely due to increased risks caused by germline *BRCA* mutations and also common hormonal risk factors. In breast cancer and AGCT, shared genetic susceptibility is not likely, as the predisposing mutations of *BRCA1* and *BRCA2* are not associated with AGCT, nor is the *FOXL2* mutation associated with breast carcinoma. A more plausible explanation lies in common risk factors such as obesity, parity, and hormonal environment. The hyperestrogenic state related to obesity is a risk factor for breast and ovarian cancer and has been suggested to

increase the risk for developing AGCT (31, 69), whereas parity is a known protective factor in both breast and ovarian cancer (20, 145, 146).

Table 12. *Risk of subsequent breast cancer among women with AGCT, and the risk of subsequent AGCT among women with breast cancer in Finland in 1968-2013.*

Risk for breast cancer after AGCT					
	Observed	**Expected**	**SIR**	**95 % CI**	**p-value**
All[1]	38	30.2	1.26	0.9-1.7	0.15
Follow-up time (years)					
0-4	6	6.9	0.87	0.34-1.76	0.73
5-14	17	12.3	1.38	0.82-2.15	0.18
>15	15	10.9	1.37	0.79-2.19	0.22
Age at AGCT diagnosis (years)					
<50	14	11.9	1.18	0.66-1.91	0.54
≥50	24	18.3	1.31	0.86-1.91	0.18
Breast cancer invasion*					
Localized	21	15.5	1.36	0.86-2.02	0.16
Non-localized	10	12.0	0.83	0.42-1.46	0.56
Risk for AGCT after breast cancer					
All[1]	25	15.7	1.59	1.04-2.29	0.02
Follow-up time (years)					
0-4	8	5.9	1.35	0.62-2.52	0.39
5-14	10	6.8	1.48	0.74-2.59	0.22
>15	7	3.1	2.28	0.98-4.41	0.03
Age at breast cancer diagnosis (y)					
<50	11	5.2	2.10	1.09-3.59	0.01
>50	14	10.5	1.33	0.75-2.16	0.28

[1]diagnosis > 6 months after primary cancer. SIR= standardized incidence ratio, CI = confidence interval.

A few case reports have suggested an association between antecendent tamoxifen use and the development of AGCT (32, 147, 148). Selective estrogen receptor modulators (SERMs), including tamoxifen, are typically used in the treatment of hormone receptor-positive breast cancer, and aromatase inhibitors, such as letrozole, are treatment options for both postmenopausal breast cancer and AGCT (100, 149). However, the relationship between breast cancer therapy and AGCT is unconfirmed, warranting further evaluation.

5.2.3 Concomitant cancers and endometrial cancer in association with AGCT

Of the 15 cancers diagnosed within six months of AGCT (SIR 5.00, 95% CI 2.80-8.23), uterine cancer accounted for 33% (n=5) (Tables 11 and 13). Other tumors included cancers of the digestive organs (n=4), breast (n=2), urinary tract (n=2), cervix uteri (n=1), and lymphoid/hematopoietic tissue (n=1). After uterine cancer, 20 women developed AGCT within six months (SIR 100.0, 95% CI 61.08-154.4). The rate for concomitant endometrial cancer can thus be approximated based on the two cancers occurring within six months of each other, resulting in altogether 25 women and a rate of 2.5%. Additionally, two patients presented with AGCT more than six months after uterine cancer (Table 13). All of the patients with subsequent AGCT after uterine cancer were aged 50 years or over at the time of primary cancer diagnosis (SIR 6.21, 95% CI 4.09-9.42).

While the presence of other tumors within a short period of AGCT is mainly explained by a strong surveillance bias, the concomitant occurrence of AGCT and endometrial cancer is associated with the effects of AGCT-derived estrogen (18, 72). The rates for concomitant endometrial cancer have been reported to lie between 5% and 11% in other population-based studies (9, 11, 13). Hospital-based series demonstrate even rates of 10-13% for endometrial carcinoma (74, 78), but clearly lower rates of 1-3% have also been described (76, 117, 130). In comparison, our rate of 2.5% is relatively low and may reflect a proportion of previously hysterectomized women. Unlike in most hospital-based and some population-based cohorts, we were not able to take into account cases with unavailable endometrial samples.

In a recent publication, a 6% rate of endometrial cancer was encountered among women with AGCT, but the risk for endometrial pathology was not increased in the median follow-up of 10 years after AGCT in patients not having undergone hysterectomy. Thus, it was reported that the risk of endometrial cancer after salpingo-oophorectomy for AGCT is even lower than in the normal population, and a routine hysterectomy was deemed unnecessary for patients with AGCT in the presence of normal endometrium (10). Ottolina et al. (2015) observed endometrial abnormalities almost exclusively in AGCT patients older than 40 years and found that no further endometrial changes occurred in patients who had undergone fertility-sparing surgery (150). Also our results indicate that endometrial cancer in association with AGCT occurs mainly in postmenopausal women, a finding also reported by Unkila-Kallio et al. (2000) based partly on the same patient cohort (73).

Although hysterectomy with salpingo-oophorectomy is a standard treatment for patients with uterine cancer, there were two cases of AGCT diagnosed after six months of uterine cancer. This may be due to the historical nature of the cohort or a case of miscoding, which cannot be further analyzed in the registry data.

Table 13. *Risk of subsequent uterine cancer among women with AGCT, and risk of subsequent AGCT among women with uterine cancer in Finland in 1968-2013.*

Risk for uterine cancer after AGCT					
	Obs	**Exp**	**SIR**	**95 % CI**	**p-value**
All	5	7.3	0.69	0.22-1.61	0.513
Diagnosis within 6 months after AGCT	5	0.2	26.02	8.45-60.72	<0.001
Diagnosis > 6 months after AGCT	0	7.1	0.00	0.00-0.52	0.013
Risk for AGCT after uterine cancer					
All	22	4.4	4.99	3.18-7.37	<0.001
Diagnosis within 6 months after uterine cancer	20	0.2	104.28	65.00-156.89	<0.001
Diagnosis > 6 months after uterine cancer	2	4.2	0.47	0.08-1.46	0.291
Age at uterine cancer diagnosis (years)					
>50	22	3.5	6.21	4.09-9.42	<0.001

Obs = observed number of cases. Exp = expected number of cases. SIR = standardized incidence ratio. CI = confidence interval.

The retrospective cohort design of the study regarding second primary malignancies has strengths and weaknesses. To our knowledge, this is the largest and most comprehensive study ever conducted in second primary malignancies among AGCT patients. The population-based registry study has a sizable number of subjects and was able to find statistically significant associations between new primary cancers among the same patients. Limitations include the lack of detailed patient information regarding, for instance, treatment for primary cancer. Additionally, in a rare cancer, such as AGCT, even a significant SIR can indicate a small absolute risk for developing a new primary malignancy.

Studies on second primary cancers might reveal previously unknown etiological factors and help in identifying subgroups of patients at increased risk for developing another cancer. This has also practical implications, as it may guide in treatment decisions regarding the first primary tumor and in planning long-term follow-up strategies after primary treatment. It is also vital for informed patient counselling. The high long-term survival rates in AGCT result in a relevant issue of possible treatment-induced cancers, where a careful evaluation of the excess risk caused by treatment modalities, such as chemotherapy, must then be performed. Also from this perspective, the development of novel, targeted, and more tolerable therapies with less side effects is one of the key challenges in the future.

5.3 Clinical characteristics and prognostic factors in AGCT (III and IV)

5.3.1 Histological and molecular re-evaluation

After histological re-evaluation in Study III, the final study cohort included 187 patients (Figure 4). This represented altogether 78% of patients with an original AGCT diagnosis. Evaluated by diagnostic eras, the rate of originally misclassified diagnoses or missing histological samples was 31% in 1956-1983 and 13% in 1984-2012. The revised diagnoses included endometrioid tumors, cellular fibromas, thecomas, metastatic carcinomas, Sertoli-Leydig cell tumors, steroid cell tumors, undifferentiated carcinomas, SCTATs, hypercalcemic small cell carcinomas, and one case each of unclassified sex cord-stromal tumor, female adnexal tumor of probable Wolffian origin (FATWO), transitiocellular carcinoma, endometrioid adenofibroma, Brenner tumor, ovarian small cell carcinoma of pulmonary type, eosinophilic clear cell carcinoma, and adenocystic carcinoma. Following the reconfirmation of AGCT diagnosis, 53% of stage III (n=8) and 100% of stage IV (n=4) tumors were excluded from the final study cohort.

In Study IV, the patient cohort was even further validated and included histologically and molecularly evaluated AGCTs with a confirmed *FOXL2* (402 C-G) mutation (Figure 5). The rates for the excluded cases were as follows: misdiagnosed cases 12%, missing or inadequate samples 15%, and negative (wild-type) *FOXL2* mutation status 5%, resulting in altogether 164 patients (68%) eligible for analysis.

In total, 83% of patients in Study cohort III were included in Study cohort IV. From the cases that were included in Study III but excluded from Study IV, 12% were negative for *FOXL2* mutation and 5% had a sample missing or inadequate for the analysis.

False-positive rates of 10-40% have been seen in other studies, where histological but not molecular re-evaluation has been performed (13, 108, 118, 130). The rates for pure misdiagnoses in our series were from 12% to 17% when missing samples were excluded. More than half of the misdiagnosed cases were carcinomas, which would have affected the assessment of AGCT prognosis. As most of the stage III-IV tumors were excluded after re-evaluation, it can be concluded that older series with relatively large numbers of high-stage tumors or tumor-related deaths probably included non-AGCT cases, as also speculated by McConechy et al. (2016) (126). Therefore, it is obvious that the accurate diagnosis of AGCT is crucial, and in the future, it is recommended that *FOXL2* mutation testing be incorporated into routine pathological assessment (126).

In Study III, the histological re-evaluation was based on the opinion of a single gynecologic pathologist and can thus be biased. In Study IV, the re-evaluation was also completed by a second expert pathologist.

5.3.2 Clinical characteristics of the patient cohort

The clinical patient characteristics of Study IV are summarized in Table 14 and tumor and treatment characteristics in Table 15. These represent largely the same patient cohort as in Study III, but with more accurate validation.

Table 14. *Clinical characteristics of histologically and molecularly defined AGCT patients.*

Characteristic	Total* (%) N=164
Year of diagnosis	
1956-1983	59 (36.0%)
1984-2014	105 (64.0%)
Age	
Median (range), years	54.4 (26-81)
<50	58 (35.4%)
>50	106 (64.6%)
Postmenopausal status	102 (63.4%)
Parity	
Nulliparous	56 (34.6%)
Primiparous	37 (22.8%)
Multiparous	69 (42.6%)
History of infertility	27 (17.3%)
Use of oral contraceptives during lifetime	54 (43.9%)
Initial symptoms	
Abnormal bleeding	76 (46.6%)
Abdominal pain	41 (25.2%)
Abdominal distension	21 (12.9%)
General symptoms (e.g. fever, weight loss)	1 (0.6%)
Asymptomatic	24 (14.7%)
Use of preoperative HRT	24 (20.0%)
Use of postoperative HRT	47 (49.0%)
*) number of patients with data available. HRT =hormone replacement therapy	

Table 15. *Primary tumor and treatment characteristics of histologically and molecularly defined AGCTs.*

Characteristic	Total* (%) N=164
FIGO stage (2014)	
I	148 (91.9%)
II	10 (6.2%)
III	3 (1.9%)
Tumor rupture	56 (35.7%)
Stage IC1	32 (57.1%)
Stage IC2	18 (32.1%)
Stage II	5 (8.9%)
Stage III	1 (1.8%)
Presence of ascites	33 (22.9%)
Tumor size	
Mean (range), cm	10.9 (0.5-30)
<10 cm	74 (47.4%)
>10 cm	82 (52.6%)
Surgical approach	
Laparoscopy	31 (18.9%)
Laparotomy	133 (81.1%)
Complete surgery (no gynecological organs left)	128 (78.5%)
Staging (peritoneal biopsies and omental biopsy or omentectomy)	48 (29.8%)
Lymphadenectomy	37 (23.0%)
Adjuvant chemotherapy	28 (17.2%)
Endometrial pathology	
Normal	52 (38.0%)
Polyps	18 (13.1%)
Hyperplasia	57 (41.6%)
Carcinoma	10 (7.3%)
*) number of patients with data available	

5.3.3 Recurrence and survival in AGCT and prognostic factors related to outcome

Recurrence (Study IV)

The mean follow-up time in the study was 16.8 years (range 1.0-51.3 years). During this period 52 patients (32%) developed at least one tumor recurrence in a median time of 7.4 years (range 1-26 years). The majority of patients experienced more than one relapse: 23

patients (44%) had one, 17 (33%) had two, five (10%) had three, four (8%) had four, two (4%) had five, and one (2%) had six tumor recurrences.

When evaluating both first and consecutive relapses, we only took into account cases where the patient was proven clinically or radiologically disease-free before the event of relapse. Thus, disease progression was not considered equal to relapse. Despite this definition, the rate of recurrence in this well-validated series was relatively high and reflects the extensive follow-up period (Table 4). The mean follow-up time of over 200 months is the longest reported, and considering the long median time for this tumor to relapse, studies with follow-up periods shorter than 80-90 months do not provide reliable data on AGCT recurrence. Only one larger study has reported a higher relapse rate for AGCT, but it included only patients from tertiary referral hospitals, where a large number of patients were only referred to after event of relapse (103). In smaller series, the relapse rates vary between 9% and 37% (114-116, 151-154).

The sites of recurrence were grouped as follows: pelvis, abdominal cavity excluding pelvis, retroperitoneum, abdominal wall, lymph nodes, bone, liver, and lung. When several recurrent tumors were simultaneously present in more than one anatomical region, the recurrence was considered multiple (Figure 10). The 52 patients with AGCT recurrence had a total of 104 events of relapse, of which 44 (42%) were multiple recurrences. The rate of multiple recurrence remained similar irrespective of whether the relapse was first, second, or higher order (44%, 41%, and 39%, respectively). Of single-site recurrences, the pelvis was the most common site of recurrence (62%), followed by abdominal cavity (20%), retroperitoneum (5%), and liver (5%). Of multiple recurrences, the pelvis and abdominal cavity were also the most common anatomical sites (34% and 17%, respectively). Tumors in the retroperitoneum, liver, bone, lung, abdominal wall, and lymph nodes were more often seen in multiple recurrences than in single-site recurrences. The site of recurrence was unknown in one case. This relatively wide variety of relapse sites supports the use of both vaginal ultrasound and CT scan in case of suspected recurrence since, despite local recurrences in the pelvis being most common, also multiple and distant tumors may develop.

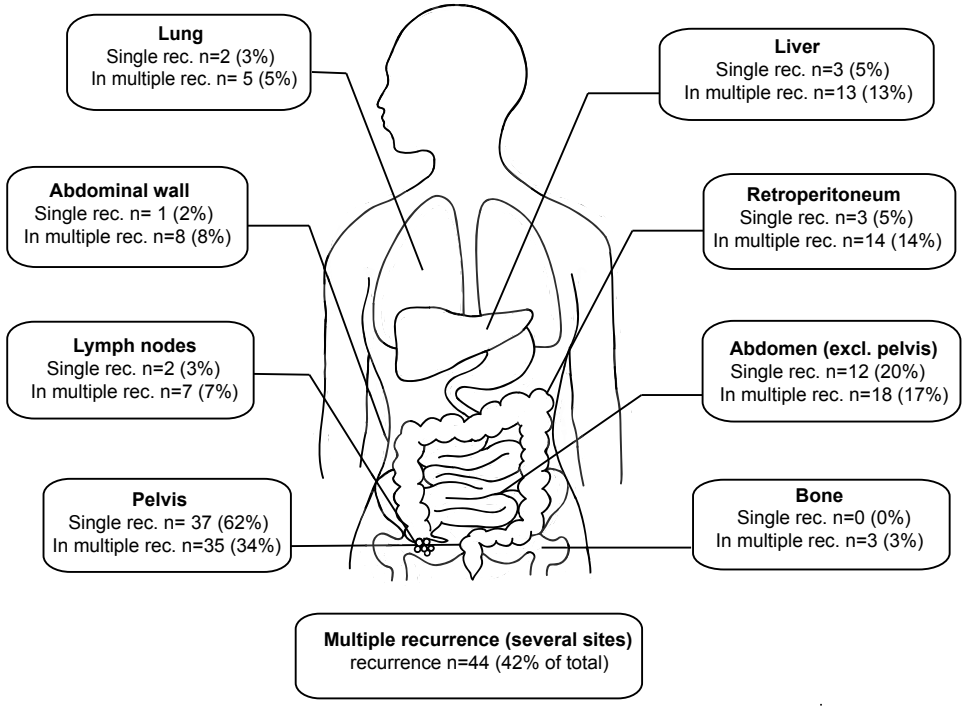

***Figure 10**. Anatomical sites of AGCT recurrence in 52 patients and 104 events of relapse. Total number of recurrent tumors is presented both as single recurrences and as part of multiple recurrences (%). Rec = recurrence.*

Survival (Study III)

The mean follow-up period in Study III was 15.6 years or 187 months (range 0.01-50 years). The outcomes are presented in Figure 11. Most patients (61%) were alive at the end of follow-up, and the rate of disease-related death was 14%. The disease-specific survival rates were as follows: 5-year survival 97%, 10-year survival 92%, and 20-year survival 87%. When comparing survival rates between the diagnostic eras in 1956-1983 and 1984-2012, the AGCT-specific 10-year survival rates increased from 88% to 95%. The overall survival rates were 94%, 87%, and 67% for 5, 10, and 20 years, respectively. Disease-related survival and recurrence were closely associated; of the relapsed patients, 44% died of AGCT.

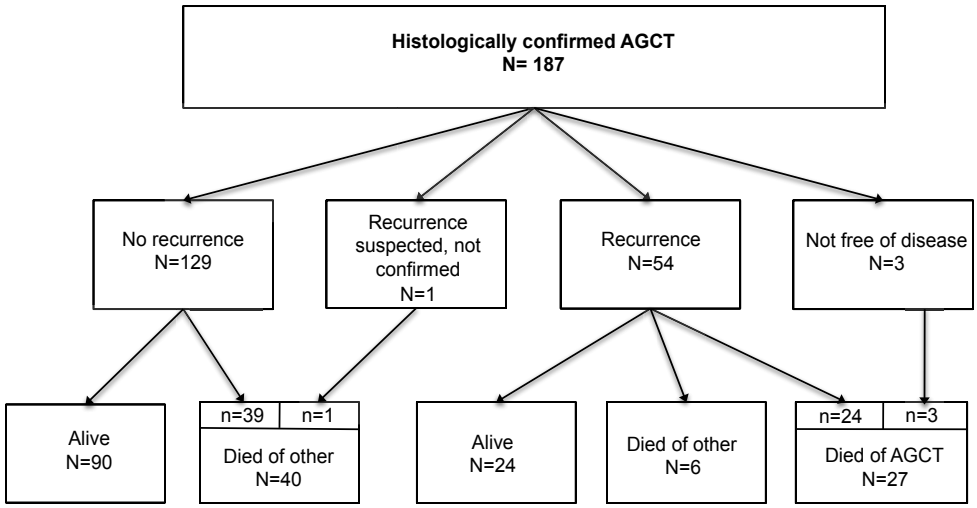

Figure 11. *Patient outcomes in Study III.*

The survival rates, particularly in the modern era, are the highest reported and confirm the generally indolent nature of AGCT. Survival has improved during the past decades, but unlike in the study by Hölscher et al. (2009), we did not observe a significant stage shift between the diagnostic eras, which was considered the main reason for improved survival in their study, which included 5% sex cord-stromal tumors other than AGCT (120). In our series, the rates of stage I tumors were 88% and 90% in the old and new era, respectively, whereas the rates for staging surgery, lymphadenectomy, and platinum-based chemotherapy increased significantly over time from 0% to 28%. This indicates that the advances in treatment, particularly the introduction of platinum-based chemotherapy, are more likely to play a role in the increased survival rates. The rates for residual tumor after primary surgery were low in both periods (3-4%). Interestingly, although the proportions of preoperative ultrasound and asymptomatic patients increased over time (16% vs. 92% and 7% vs. 18%, respectively), this did not seem to have an impact on the stage distribution between the eras. Between the diagnostic periods, tumor size and age at diagnosis remained similar, whereas infertility, use of oral contraceptives, use of hormone replacement therapy, and tumor rupture were more common in 1984-2012. The recurrence rates were 36% in the old era and 25% in the new era, but this difference was not statistically significant (p=0.11).

Prognostic factors (Studies III and IV)

The prognostic factors were evaluated for an association with AGCT relapse (Study IV) and disease-related death (Study III). In cross-tabulation analysis, there were more relapses among premenopausal than postmenopausal women at the time of primary diagnosis (p=0.004), and younger age was associated with relapse as both a categorical (<50 years) and a continuous variable (p=0.008 and p=0.046, respectively). Tumor ruptures (p=0.005), lack of staging surgery (p=0.002), and lack of lymphadenectomy (p=0.047) were more often seen among patients with recurrence. The two latter characteristics were also associated with relapse in a subanalysis of Stage IC tumors. The year of diagnosis (the "diagnostic era"), the use of oral contraceptives or HRT, surgical approach, tumor size, or FIGO stage had no significant effect on recurrence.

The results were similar when evaluating prognostic factors in Kaplan-Meier and Cox univariate analyses; the risk of AGCT recurrence was increased in premenopausal patients, FIGO stage IC versus IA, and in patients with tumor rupture (Figure 12). The prognostic effect of tumor rupture was significant in both IC1 (intraoperative) and IC2 (preoperative) ruptures. In Cox multivariate analysis, tumor rupture was the only independent risk factor for AGCT relapse. This result was further confirmed when evaluating risk factors for successive relapses after first relapse; tumor rupture was the only significant risk factor in this analysis.

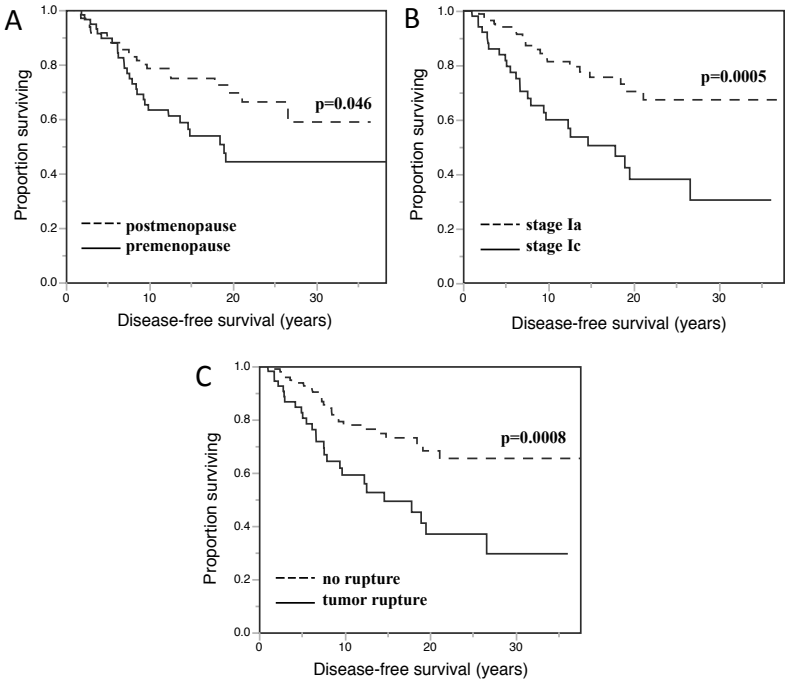

Figure 12. *Kaplan-Meier curves of disease-free survival. A. Menopausal status. B. Stage Ia vs Stage Ic. C. Presence of tumor rupture*

In survival analysis, univariate models showed an increased risk for disease-specific death in the diagnostic period of 1956-1983, for patients aged over 60 years, larger tumor size, advanced stage, presence of residual tumor, and use of hormonal adjuvant therapy (Table 16). Infertility and the use of oral contraceptives were associated with better prognosis. However, advanced stage was the only independent prognostic factor for AGCT-related death in multivariate analysis.

Table 16. *Cox regression analyses for AGCT-specific survival.*

Univariate analysis				**Multivariate analysis**		
Factor	**HR**	**95 % CI**	**P**	**HR**	**95 % CI**	**P**
Infertility	0.20	0.01-0.93	0.04	0.80	0.04-5.56	ns
Use of OC	0.33	0.08-0.99	0.05	0.85	0.16-3.77	ns
Stage II-III vs Stage I	16.19	6.26-43.42	<0.0001	10.51	1.65-69.48	0.01
Residual tumor	10.98	3.09-31.08	0.0009	1.71	0.18-13.97	ns
Hormonal adjuvant	3.64	1.33-8.52	0.02	3.33	0.80-12.37	ns
Age >60 years	2.82	1.16-6.73	0.02	1.07	0.20-4.90	ns
Diagnosis in 1956-1983	2.68	1.07-7.63	0.04	2.17	0.40-18.44	ns
Tumor size	2.43	1.02-6.68	0.04	3.10	0.75-21.07	ns
HR=hazard ratio. CI=confidence interval. OC=oral contraceptives. ns=not significant.						

In Kaplan-Meier analysis, presence of residual tumor and advanced stage were the strongest predictive factors for disease-related survival (Figure 13). Age over 60 years and earlier diagnostic period were also significantly associated with poor survival. The use of non-platinum-based chemotherapy versus platinum-based chemotherapy, and tumor size equal to or over 10 cm were marginally significant (p=0.05, data not shown). To further evaluate the effect on platinum-based chemotherapy, AGCT-specific survival was analyzed between the eras after exclusion of patients who received chemotherapy, and no significant difference was observed between these groups. Interestingly, although tumor rupture was a significant prognostic factor for AGCT recurrence, it did not affect survival in our analyses.

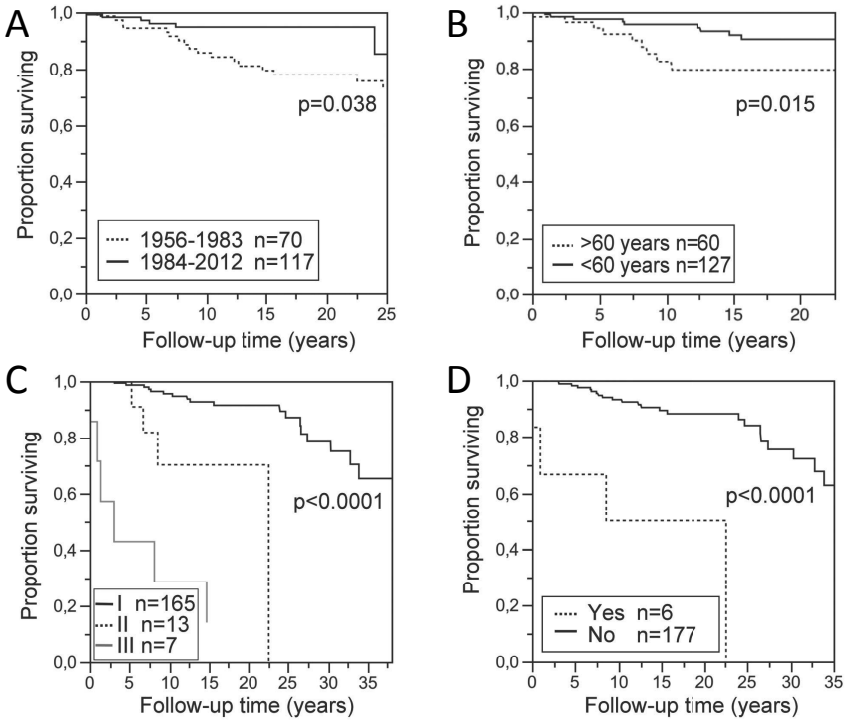

***Figure 13**. Kaplan-Meier curves of AGCT-specific survival. A. Year of diagnosis. B. Age at diagnosis. C. Tumor stage D. Presence of residual disease after primary treatment.*

The value of analyzing prognostic factors related to disease outcome is in identifying the patients at increased risk for tumor relapse or tumor-related death, and in guiding treatment decisions and patient counseling based on these data. Our results indicate that patients with stage IC disease or tumor rupture should be considered as high risk with regard to AGCT recurrence, and patients with advanced stage (II-III) and residual tumor after primary treatment have worse prognosis in terms of disease-related death, and thus are likely to benefit from adjuvant treatment following surgery. Furthermore, it seems evident that the introduction of platinum-based chemotherapy has improved survival among women with AGCT. The use of hormonal adjuvant therapy in this series was more common in the old era (17.1% vs. 0.9%), typically consisting of a high-dose medroxyprogesterone acetate. Thus, the poor prognostic effect of hormonal adjuvant treatment in univariate analysis is most likely explained by the treatment era.

5.3.4 Management and follow-up of AGCT

After the diagnosis and primary treatment for AGCT in HUH, the mean time in the hospital-based clinical routine follow-up was 5.5 years with a median of 11 visits. There was no statistical difference in the follow-up depending on whether or not the patient developed tumor recurrence or not (6.2 and 5.4 years, respectively). The clinical follow-up consisted of gynecologic examination together with determination of serum markers (71%), transabdominal or transvaginal pelvic ultrasound (68%), and a CT scan (28%). The diagnostic era had a natural impact on the follow-up protocol, where routine monitoring for inhibin B began in 1998 and for AMH in 2014. Either one of these markers was measured in the primary follow-up of a total of 70 patients (49%).

Three-fourths (75%) of the first disease relapses occurred within ten years after primary diagnosis, and almost 90% within 15 years (Figure 14). In patients with more than one event of relapse, the median time between the first and second relapse was 2.9 years, and between the second and third 1.4 years. This pattern is similar to that of epithelial ovarian cancer, where the median time between consecutive relapses shortens with each relapse, supporting the theory that the likelihood for any malignancy to accumulate mutations and develop drug resistance increases over time (155).

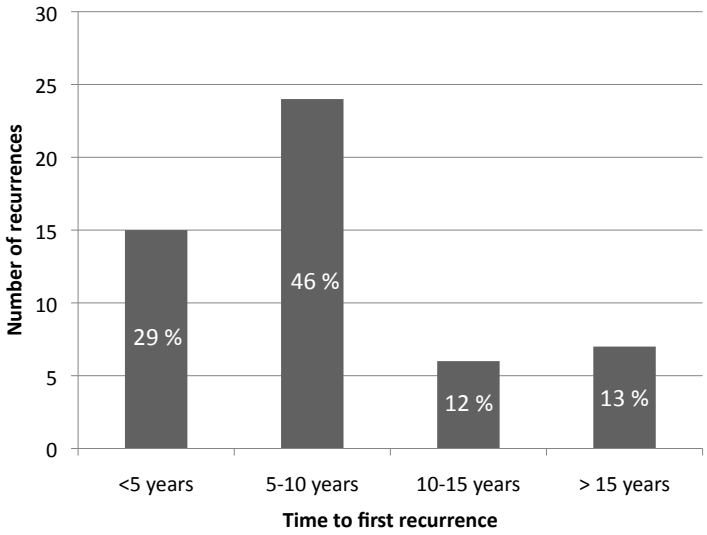

Figure 14. *Time to first AGCT relapse, by five-year intervals.*

Upon relapse, the proportion of asymptomatic tumors was remarkable; 38% of first recurrences and 57-62% of higher order recurrences were detected without presenting symptoms. Nearly half (45%) of these asymptomatic tumors were local pelvic recurrences. Inhibin B was elevated in 94% of relapses when measured, whereas Ca-125 was not as sensitive (33%). A total of 83% of first recurrences and 57-59% of second or higher order recurrences were treated either by radical surgery alone or with a combination of surgery and adjuvant therapy (chemo- or radiotherapy). The proportion of chemotherapy as a sole treatment increased from 6% in first recurrences to 17% in third or higher order recurrences. Other, less common treatment options included palliative surgery, radiotherapy alone or in combination with chemotherapy, aromatase inhibitors, or bevacizumab.

These results indicate that the routine follow-up after AGCT should be at least ten years after primary diagnosis and treatment and should not be based on clinical symptoms only as recurrences are often asymptomatic. Also, as even several consecutive recurrences may be radically treated by surgery or with a combination or surgery and chemotherapy, the early detection seems beneficial. However, it is still unclear whether the detection of asymptomatic recurrences actually improves patient survival or quality of life; in our series, the median survival was over 25 years irrespective of whether tumor recurrence developed or not, and the recent study by McConechy et al. (2016) showed no statistical difference in overall survival of AGCT patients with and without recurrence relative to an age-matched control population for the first ten years (126). This reflects the indolent course of AGCT also in a recurrent setting, despite the increased proportion of disease-related death among these patients.

The findings from the survival analysis support the use of platinum-based chemotherapy. We did not see a significant difference in survival related to staging surgery or performance of lymphadenectomy in the primary setting, but the lack of staging surgery, lymphadenectomy, and adjuvant chemotherapy were associated with disease recurrence, particularly among stage IC patients. This suggests that this patient group may benefit from a more aggressive treatment approach. Furthermore, younger age and premenopausal status at primary diagnosis were associated with recurrence in the cross tabulation analysis, which partly reflects the longer follow-up period, but also partly supports the suggestion that radical surgery should be considered when pregnancy is no longer desired among women who had initially undergone fertility-sparing procedures.

Moreover, we found that the use of postmenopausal hormonal therapy did not affect AGCT-related survival or development of tumor recurrence. This is a significant finding since AGCT typically affects women in the perimenopausal or early postmenopausal period, and radical surgery may lead to an acute onset of possibly severe menopausal symptoms. The use of HRT in ovarian cancer survivors lacks solid evidence-based guidelines and is challenged by the fear of recurrent disease, although there is very little evidence that hormone therapy is contraindicated in these women (156). A rather recent review concluded that survivors of estrogen-related gynecological cancers, such as

AGCTs, should not be offered HRT, but no evidence supports this statement (157). There are no randomized studies (RCTs) concerning the use of HRT among AGCT survivors, and only two small RCTs concerning the use of HRT after epithelial ovarian cancer (158, 159). In these studies, 75 and 125 patients were randomized to HRT or non-HRT groups following cancer surgery, respectively, with no significant differences in age, tumor histology or differentiation, clinical stage, or treatment between HRT and non-HRT groups, and postoperative HRT did not have a negative effect on prognosis. Furthermore, two recent reviews have concluded that estrogen therapy does not impact negatively on the outcome of ovarian cancer patients, and thus, it can be considered as a therapeutic option when needed (157, 160). A number of studies have even shown a favorable outcome with HRT use following invasive epithelial or borderline ovarian cancer relative to non-use (161-163). It must be recognized, however, that these observational cohort studies may suffer from selection and/or publication bias. As a whole, the current data suggest that the use of HRT does not worsen the prognosis of ovarian cancer survivors, but larger RCTs are needed to verify this finding.

In these studies based on single-institute (HUH) patient cohorts, we have summarized the clinical picture and prognostic factors of this rare cancer using well-validated and comparatively large patient series. In addition to histological and molecular validation, study strenghts include the long follow-up periods of several decades, which provide reliable information on late-recurring tumors such as AGCTs. The long time span allows observations of the developments in diagnostic and treatment modalities over the years, which also leads to inconsistencies within patient cohorts. The inclusion of both retrospective and prospective patient cohorts may lead to potential bias, as the prospective patient group undergoes prolonged clinical surveillance, and more detailed clinical information is available for this subgroup.

A summary of the management and follow-up of AGCT is assembled based on our results (Figure 15). In the primary setting, this mainly follows the lines of the NCCN recommendation (Figure 3). Furthermore, we have included the surveillance recommendation after primary treatment.

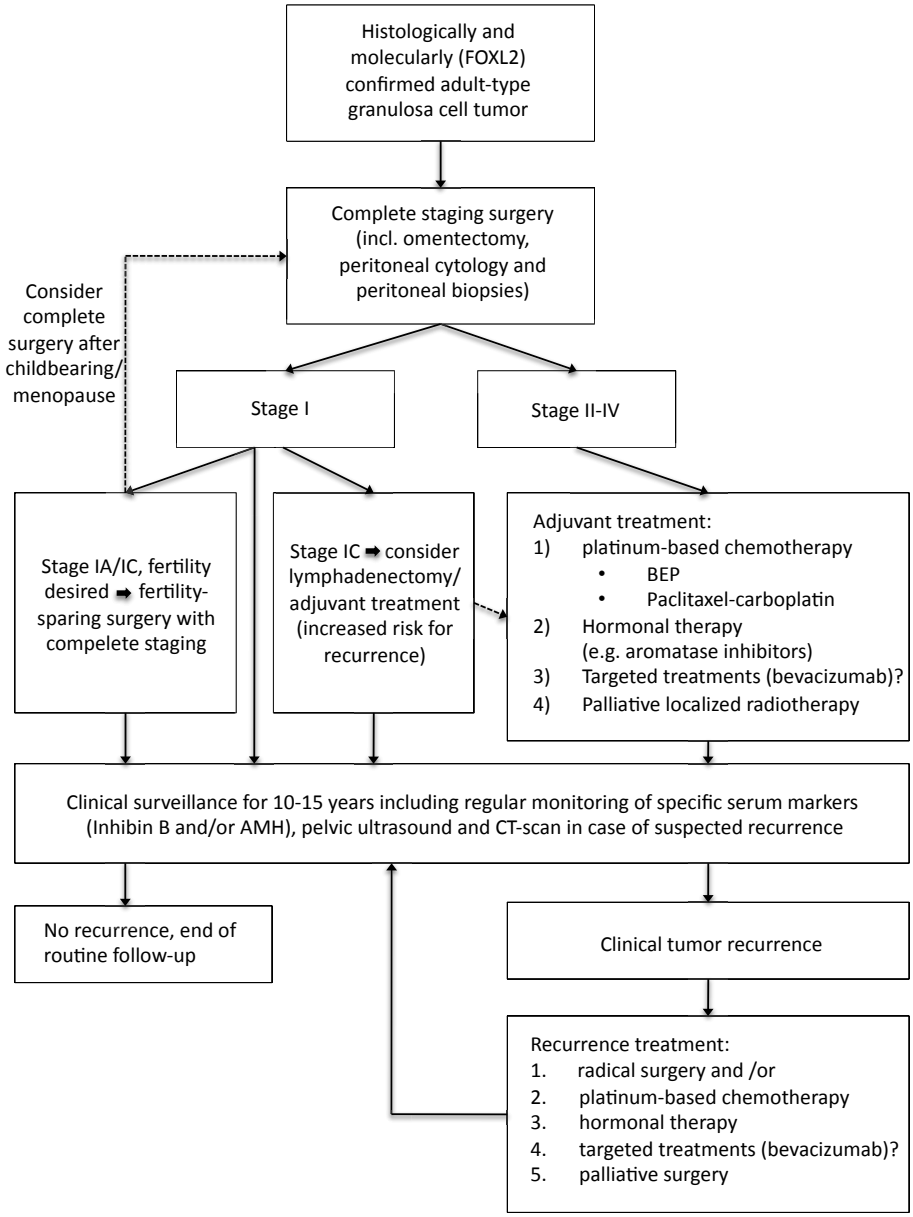

***Figure 15**. Summary and recommendation for AGCT management and follow-up.*

6 Conclusions and future prospects

Our results show the following:

1. During the study period of 1953-2012 the incidence of AGCT was relatively stable, and no significant differences emerged between the four Nordic countries examined. The age-specific incidence was highest among peri- and postmenopausal women aged 50-64 years. No occupational category was associated with an increased risk for developing AGCT. These findings indicate that AGCT is a primarily sporadic cancer.

2. AGCT patients had an increased risk for subsequent soft tissue and thyroid cancers and leukemia, most likely related to both shared risk factors and therapy-induced side effects. Women with a first primary breast cancer were at increased risk of developing subsequent AGCT, and the risk was over 2-fold in breast cancer patients younger than 50 years at the time of primary diagnosis. This may indicate shared hormonal etiology between breast cancer and AGCT.

3. The histological diagnosis of AGCT is challenging and prone to errors. The disease-specific survival rates of AGCT are excellent, particularly since the introduction of modern imaging techniques and platinum-based chemotherapy. Tumor stage is the only independent prognostic factor related to AGCT-specific survival.

4. In a molecularly validated patient cohort, tumor rupture was the strongest predictive factor for AGCT relapse. AGCT requires at least 10 years of clinical follow-up based on specific serum markers, and imaging in case of suspected recurrence.

This is the most comprehensive study on AGCT incidence to date and the first one to assess occupational risks for this rare disease. AGCT may have environmental, hormonal, and lifestyle-related etiological factors, which are difficult to identify in registry-based analyses. In the future, larger multinational studies that are also able to adjust for confounding factors regarding e.g. reproductive factors are preferable in detecting potential associations and assessing risk factors in the development of AGCT. More specifically, an epidemiological analysis regarding the use of HRT both before and after AGCT would be required to provide more solid evidence to support clinical guidance and decision-making.

Future research on second primary malignancies should seek means to identify patients at increased risk for therapy-induced cancers and to reduce the effect of common risk factors such as obesity. For these patients, alternative or modified management strategies could be considered, and the unraveling of the molecular pathogenesis of AGCT will hopefully lead to advances in developing novel, more effective and tolerable targeted therapies for this rare disease. The risk for second malignancy must be noted in both clinical follow-up and

patient counseling, especially among endocrine-related cancers such as AGCT and breast cancer.

Our analysis based on histologically and molecularly defined AGCTs confirmed the clinical risk factors related to AGCT recurrence and disease-specific survival. These results help in identifying high-risk patients who may benefit from more aggressive treatment approaches and intensive follow-up. Our findings provide a more evidence-based and detailed platform for clinical surveillance strategies. However, future studies call for 1) randomized, prospective data regarding the role of adjuvant therapy in AGCT and for stage IC patients specifically, and 2) quality-of-life and survival analyses regarding the early detection of AGCT recurrence. As the data collection for the prospective series in our study continues, we are hoping to find answers also to these questions.

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References

1. Chen VW, Ruiz B, Killeen JL, Cote TR, Wu XC, Correa CN. Pathology and classification of ovarian tumors. Cancer. 2003;97(10 Suppl):2631-42.
2. Mutch DG, Prat J. 2014 FIGO staging for ovarian, fallopian tube and peritoneal cancer. Gynecol Oncol. 2014;133(3):401-4.
3. Kurman RJ CM, Herrington CS, editor. WHO Classification of Tumours of Female Reproductive Organs. 4 ed: WHO Press; 2014.
4. Fuller PJ, Leung D, Chu S. Genetics and genomics of ovarian sex cord-stromal tumors. Clin Genet. 2017;91(2):285-91.
5. Jamieson S, Fuller PJ. Molecular pathogenesis of granulosa cell tumors of the ovary. Endocrine reviews. 2012;33(1):109-44.
6. Schumer ST, Cannistra SA. Granulosa cell tumor of the ovary. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21(6):1180-9.
7. Schultz KA, Harris AK, Schneider DT, Young RH, Brown J, Gershenson DM, et al. Ovarian Sex Cord-Stromal Tumors. J Oncol Pract. 2016;12(10):940-6.
8. McGuire S. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Adv Nutr. 2016;7(2):418-9.
9. Björkholm E, Silfverswärd C. Granulosa- and theca-cell tumors. Incidence and occurrence of second primary tumors. Acta Radiol Oncol. 1980;19(3):161-7.
10. van Meurs HS, Bleeker MC, van der Velden J, Overbeek LI, Kenter GG, Buist MR. The incidence of endometrial hyperplasia and cancer in 1031 patients with a granulosa cell tumor of the ovary: long-term follow-up in a population-based cohort study. Int J Gynecol Cancer. 2013;23(8):1417-22.
11. Ohel G, Kaneti H, Schenker JG. Granulosa cell tumors in Israel: a study of 172 cases. Gynecol Oncol. 1983;15(2):278-86.
12. Unkila-Kallio L, Leminen A, Tiitinen A, Ylikorkala O. Nationwide data on falling incidence of ovarian granulosa cell tumours concomitant with increasing use of ovulation inducers. Hum Reprod. 1998;13(1O):2828-30.
13. Lauszus FF, Petersen AC, Neumann G, Cleemann L, Rosgaard A, Jorgensen A, et al. Less extensive surgery compared to extensive surgery: survival seems similar in young women with adult ovarian granulosa cell tumor. European journal of obstetrics, gynecology, and reproductive biology. 2014;177:61-6.
14. Farkkila A, Haltia UM, Tapper J, McConechy MK, Huntsman DG, Heikinheimo M. Pathogenesis and treatment of adult-type granulosa cell tumor of the ovary. Ann Med. 2017:1-13.
15. Shah SP, Köbel M, Senz J, Morin RD, Clarke BA, Wiegand KC, et al. Mutation of FOXL2 in granulosa-cell tumors of the ovary. N Engl J Med. 2009;360(26):2719-29.
16. Jamieson S, Butzow R, Andersson N, Alexiadis M, Unkila-Kallio L, Heikinheimo M, et al. The FOXL2 C134W mutation is characteristic of adult granulosa cell tumors of the ovary. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2010;23(11):1477-85.
17. Rosario R, Cohen PA, Shelling AN. The role of FOXL2 in the pathogenesis of adult ovarian granulosa cell tumours. Gynecol Oncol. 2014;133(2):382-7.
18. Stine JE, Pierce S, Soper JT. A comprehensive review of diagnostic and treatment options for granulosa cell tumors of the ovary. Obstetrical & gynecological survey. 2014;69(1):29-38.
19. Stevens TA, Brown J, Zander DS, Bevers MW, Gershenson DM, Ramondetta LM. Adult granulosa cell tumors of the ovary in two first-degree relatives. Gynecol Oncol. 2005;98(3):502-5.
20. Adami HO, Hsieh CC, Lambe M, Trichopoulos D, Leon D, Persson I, et al. Parity, age at first childbirth, and risk of ovarian cancer. Lancet. 1994;344(8932):1250-4.

21. Collaborative Group on Epidemiological Studies of Ovarian C, Beral V, Doll R, Hermon C, Peto R, Reeves G. Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls. Lancet. 2008;371(9609):303-14.
22. Le ND, Leung A, Brooks-Wilson A, Gallagher RP, Swenerton KD, Demers PA, et al. Occupational exposure and ovarian cancer risk. Cancer Causes Control. 2014;25(7):829-41.
23. Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. IV. The pathogenesis of epithelial ovarian cancer. Collaborative Ovarian Cancer Group. Am J Epidemiol. 1992;136(10):1212-20.
24. Casagrande JT, Louie EW, Pike MC, Roy S, Ross RK, Henderson BE. "Incessant ovulation" and ovarian cancer. Lancet. 1979;2(8135):170-3.
25. Tortolero-Luna G, Mitchell MF. The epidemiology of ovarian cancer. J Cell Biochem Suppl. 1995;23:200-7.
26. Shi LF, Wu Y, Li CY. Hormone therapy and risk of ovarian cancer in postmenopausal women: a systematic review and meta-analysis. Menopause. 2016;23(4):417-24.
27. Collaborative Group On Epidemiological Studies Of Ovarian C, Beral V, Gaitskell K, Hermon C, Moser K, Reeves G, et al. Menopausal hormone use and ovarian cancer risk: individual participant meta-analysis of 52 epidemiological studies. Lancet. 2015;385(9980):1835-42.
28. Koskela-Niska V, Lyytinen H, Riska A, Pukkala E, Ylikorkala O. Ovarian cancer risk in postmenopausal women using estradiol-progestin therapy - a nationwide study. Climacteric. 2013;16(1):48-53.
29. Horn-Ross PL, Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 U.S. case-control studies. VI. Nonepithelial cancers among adults. Collaborative Ovarian Cancer Group. Epidemiology. 1992;3(6):490-5.
30. Schuler S, Ponnath M, Engel J, Ortmann O. Ovarian epithelial tumors and reproductive factors: a systematic review. Arch Gynecol Obstet. 2013;287(6):1187-204.
31. Boyce EA, Costaggini I, Vitonis A, Feltmate C, Muto M, Berkowitz R, et al. The epidemiology of ovarian granulosa cell tumors: a case-control study. Gynecol Oncol. 2009;115(2):221-5.
32. Abahssain H, Kairouani M, Gherman R, M'Rabti H, Errihani H. Granulosa cell tumor of the ovary and antecedent of adjuvant tamoxifen use for breast cancer. World journal of surgical oncology. 2010;8:67.
33. Venn A, Healy D, McLachlan R. Cancer risks associated with the diagnosis of infertility. Best Pract Res Clin Obstet Gynaecol. 2003;17(2):343-67.
34. Trabert B, Lamb EJ, Scoccia B, Moghissi KS, Westhoff CL, Niwa S, et al. Ovulation-inducing drugs and ovarian cancer risk: results from an extended follow-up of a large United States infertility cohort. Fertil Steril. 2013;100(6):1660-6.
35. Sieh W, Sundquist K, Sundquist J, Winkleby MA, Crump C. Intrauterine factors and risk of nonepithelial ovarian cancers. Gynecol Oncol. 2014;133(2):293-7.
36. Chen T, Surcel HM, Lundin E, Kaasila M, Lakso HA, Schock H, et al. Circulating sex steroids during pregnancy and maternal risk of non-epithelial ovarian cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2011;20(2):324-36.
37. Kliewer EV, Smith KR. Ovarian cancer mortality among immigrants in Australia and Canada. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 1995;4(5):453-8.
38. Parkin DM, Iscovich J. Risk of cancer in migrants and their descendants in Israel: II. Carcinomas and germ-cell tumours. Int J Cancer. 1997;70(6):654-60.

39. Mink PJ, Sherman ME, Devesa SS. Incidence patterns of invasive and borderline ovarian tumors among white women and black women in the United States. Results from the SEER Program, 1978-1998. Cancer. 2002;95(11):2380-9.
40. Pukkala E, Martinsen JI, Lynge E, Gunnarsdottir HK, Sparén P, Tryggvadottir L, et al. Occupation and cancer - follow-up of 15 million people in five Nordic countries. Acta Oncol. 2009;48(5):646-790.
41. Camargo MC, Stayner LT, Straif K, Reina M, Al-Alem U, Demers PA, et al. Occupational exposure to asbestos and ovarian cancer: a meta-analysis. Environ Health Perspect. 2011;119(9):1211-7.
42. Lie JA, Andersen A, Kjaerheim K. Cancer risk among 43000 Norwegian nurses. Scand J Work Environ Health. 2007;33(1):66-73.
43. King AS, Threlfall WJ, Band PR, Gallagher RP. Mortality among female registered nurses and school teachers in British Columbia. Am J Ind Med. 1994;26(1):125-32.
44. Sala M, Dosemeci M, Zahm SH. A death certificate-based study of occupation and mortality from reproductive cancers among women in 24 US states. J Occup Environ Med. 1998;40(7):632-9.
45. MacArthur AC, Le ND, Abanto ZU, Gallagher RP. Occupational female breast and reproductive cancer mortality in British Columbia, Canada, 1950-94. Occup Med (Lond). 2007;57(4):246-53.
46. Petralia SA, Dosemeci M, Adams EE, Zahm SH. Cancer mortality among women employed in health care occupations in 24 U.S. states, 1984-1993. Am J Ind Med. 1999;36(1):159-65.
47. Vasama-Neuvonen K, Pukkala E, Paakkulainen H, Mutanen P, Weiderpass E, Boffetta P, et al. Ovarian cancer and occupational exposures in Finland. Am J Ind Med. 1999;36(1):83-9.
48. Boffetta P, Andersen A, Lynge E, Barlow L, Pukkala E. Employment as hairdresser and risk of ovarian cancer and non-Hodgkin's lymphomas among women. J Occup Med. 1994;36(1):61-5.
49. Spinelli JJ, Gallagher RP, Band PR, Threlfall WJ. Multiple myeloma, leukemia, and cancer of the ovary in cosmetologists and hairdressers. Am J Ind Med. 1984;6(2):97-102.
50. Teta MJ, Walrath J, Meigs JW, Flannery JT. Cancer incidence among cosmetologists. Journal of the National Cancer Institute. 1984;72(5):1051-7.
51. Straif K, Baan R, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, et al. Carcinogenicity of shift-work, painting, and fire-fighting. Lancet Oncol. 2007;8(12):1065-6.
52. Bhatti P, Cushing-Haugen KL, Wicklund KG, Doherty JA, Rossing MA. Nightshift work and risk of ovarian cancer. Occup Environ Med. 2013;70(4):231-7.
53. Shen N, Weiderpass E, Antilla A, Goldberg MS, Vasama-Neuvonen KM, Boffetta P, et al. Epidemiology of occupational and environmental risk factors related to ovarian cancer. Scand J Work Environ Health. 1998;24(3):175-82.
54. Shields T, Gridley G, Moradi T, Adami J, Plato N, Dosemeci M. Occupational exposures and the risk of ovarian cancer in Sweden. Am J Ind Med. 2002;42(3):200-13.
55. Wernli KJ, Ray RM, Gao DL, Fitzgibbons ED, Camp JE, Astrakianakis G, et al. Occupational exposures and ovarian cancer in textile workers. Epidemiology. 2008;19(2):244-50.
56. Guo J, Kauppinen T, Kyyronen P, Heikkila P, Lindbohm ML, Pukkala E. Risk of esophageal, ovarian, testicular, kidney and bladder cancers and leukemia among finnish workers exposed to diesel or gasoline engine exhaust. Int J Cancer. 2004;111(2):286-92.
57. Charbotel B, Fervers B, Droz JP. Occupational exposures in rare cancers: A critical review of the literature. Critical reviews in oncology/hematology. 2014;90(2):99-134.

58. Travis LB, Demark Wahnefried W, Allan JM, Wood ME, Ng AK. Aetiology, genetics and prevention of secondary neoplasms in adult cancer survivors. Nat Rev Clin Oncol. 2013;10(5):289-301.
59. Travis LB, Curtis RE, Boice JD, Jr., Platz CE, Hankey BF, Fraumeni JF, Jr. Second malignant neoplasms among long-term survivors of ovarian cancer. Cancer research. 1996;56(7):1564-70.
60. Hung YP, Liu CJ, Hu YW, Chen MH, Li CP, Yeh CM, et al. Secondary Primary Malignancy Risk in Patients With Ovarian Cancer in Taiwan: A Nationwide Population-Based Study. Medicine. 2015;94(38):e1626.
61. Shimada T, Saito T, Okadome M, Shimamoto K, Ariyoshi K, Eto T, et al. Secondary leukemia after chemotherapy and/or radiotherapy for gynecologic neoplasia. Int J Gynecol Cancer. 2014;24(2):178-83.
62. Kehoe S, Powell JE. Prior, synchronous and secondary malignancies in women with ovarian cancer. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2001;73(3):265-7.
63. Dent SF, Klaassen D, Pater JL, Zee B, Whitehead M. Second primary malignancies following the treatment of early stage ovarian cancer: update of a study by the National Cancer Institute of Canada--Clinical Trials Group (NCIC-CTG). Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2000;11(1):65-8.
64. Bergfeldt K, Silfversward C, Einhorn S, Hall P. Overestimated risk of second primary malignancies in ovarian cancer patients. European journal of cancer. 2000;36(1):100-5.
65. Bouchardy C, Fernandez S, Merglen A, Usel M, Fioretta G, Rapiti E, et al. Increased risk of second cancer among patients with ovarian borderline tumors. Gynecol Oncol. 2008;109(2):210-4.
66. Kaldor JM, Day NE, Band P, Choi NW, Clarke EA, Coleman MP, et al. Second malignancies following testicular cancer, ovarian cancer and Hodgkin's disease: an international collaborative study among cancer registries. Int J Cancer. 1987;39(5):571-85.
67. Nakonechny QB, Gilks CB. Ovarian Cancer in Hereditary Cancer Susceptibility Syndromes. Surgical pathology clinics. 2016;9(2):189-99.
68. Hunn J, Rodriguez GC. Ovarian cancer: etiology, risk factors, and epidemiology. Clin Obstet Gynecol. 2012;55(1):3-23.
69. Kyrgiou M, Kalliala I, Markozannes G, Gunter MJ, Paraskevaidis E, Gabra H, et al. Adiposity and cancer at major anatomical sites: umbrella review of the literature. BMJ. 2017;356:j477.
70. Travis LB. The epidemiology of second primary cancers. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15(11):2020-6.
71. Hammer A, Lauszus FF, Petersen AC. Ovarian granulosa cell tumor and increased risk of breast cancer. Acta Obstet Gynecol Scand. 2013;92(12):1422-5.
72. Antolic ZN, Kovacic J, Rainer S. Theca and granulosa cell tumors and endometrial adenocarcinoma. Gynecol Oncol. 1980;10(3):273-8.
73. Unkila-Kallio L, Tiitinen A, Wahlström T, Lehtovirta P, Leminen A. Reproductive features in women developing ovarian granulosa cell tumour at a fertile age. Hum Reprod. 2000;15(3):589-93.
74. Evans AT, 3rd, Gaffey TA, Malkasian GD, Jr., Annegers JF. Clinicopathologic review of 118 granulosa and 82 theca cell tumors. Obstetrics and gynecology. 1980;55(2):231-8.
75. Meisel JL, Hyman DM, Jotwani A, Zhou Q, Abu-Rustum NR, Iasonos A, et al. The role of systemic chemotherapy in the management of granulosa cell tumors. Gynecol Oncol. 2015;136(3):505-11.

76. Ayhan A, Salman MC, Velipasaoglu M, Sakinci M, Yuce K. Prognostic factors in adult granulosa cell tumors of the ovary: a retrospective analysis of 80 cases. J Gynecol Oncol. 2009;20(3):158-63.
77. Wilson MK, Fong P, Mesnage S, Chrystal K, Shelling A, Payne K, et al. Stage I granulosa cell tumours: A management conundrum? Results of long-term follow up. Gynecol Oncol. 2015;138(2):285-91.
78. Fox H, Agrawal K, Langley FA. A clinicopathologic study of 92 cases of granulosa cell tumor of the ovary with special reference to the factors influencing prognosis. Cancer. 1975;35(1):231-41.
79. Sun HD, Lin H, Jao MS, Wang KL, Liou WS, Hung YC, et al. A long-term follow-up study of 176 cases with adult-type ovarian granulosa cell tumors. Gynecol Oncol. 2012;124(2):244-9.
80. Robinson JB, Im DD, Logan L, McGuire WP, Rosenshein NB. Extraovarian granulosa cell tumor. Gynecol Oncol. 1999;74(1):123-7.
81. Paul PC, Chakraborty J, Chakrabarti S, Chattopadhyay B. Extraovarian granulosa cell tumor. Indian J Pathol Microbiol. 2009;52(2):231-3.
82. Kim SH, Park HJ, Linton JA, Shin DH, Yang WI, Chung WY, et al. Extraovarian granulosa cell tumor. Yonsei Med J. 2001;42(3):360-3.
83. Stine JE, Suri A, Gehrig PA, Chiu M, Erickson BK, Huh WK, et al. Pre-operative imaging with CA125 is a poor predictor for granulosa cell tumors. Gynecol Oncol. 2013;131(1):59-62.
84. Kim JA, Chun YK, Moon MH, Lee YH, Cho HC, Lee MS, et al. High-resolution sonographic findings of ovarian granulosa cell tumors: correlation with pathologic findings. J Ultrasound Med. 2010;29(2):187-93.
85. Huang YT, Lee JC, Kumar AS. Variable F-18 fluorodeoxyglucose avidity of metastatic recurrent adult granulosa cell tumor. Clinical nuclear medicine. 2009;34(10):710-2.
86. Caoduro C, Ungureanu CM, Singeorzan CM, Angoue O, Blagosklonov O, Boulahdour H. Granulosa cell tumor of the ovary with high FDG uptake. Clinical nuclear medicine. 2013;38(7):553-6.
87. Raj G, Proietto A, Jaaback K. Positron emission tomography and granulosa cell tumor recurrence: a report of 2 cases. Int J Gynecol Cancer. 2009;19(9):1542-4.
88. Haltia UM, Hallamaa M, Tapper J, Hynninen J, Alfthan H, Kalra B, et al. Roles of human epididymis protein 4, carbohydrate antigen 125, inhibin B and anti-Mullerian hormone in the differential diagnosis and follow-up of ovarian granulosa cell tumors. Gynecol Oncol. 2017;144(1):83-9.
89. Geerts I, Vergote I, Neven P, Billen J. The role of inhibins B and antimullerian hormone for diagnosis and follow-up of granulosa cell tumors. Int J Gynecol Cancer. 2009;19(5):847-55.
90. Rosner W, Hankinson SE, Sluss PM, Vesper HW, Wierman ME. Challenges to the measurement of estradiol: an endocrine society position statement. J Clin Endocrinol Metab. 2013;98(4):1376-87.
91. Al-Agha OM, Huwait HF, Chow C, Yang W, Senz J, Kalloger SE, et al. FOXL2 is a sensitive and specific marker for sex cord-stromal tumors of the ovary. The American journal of surgical pathology. 2011;35(4):484-94.
92. Colombo N, Peiretti M, Garbi A, Carinelli S, Marini C, Sessa C, et al. Non-epithelial ovarian cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2012;23 Suppl 7:vii20-6.
93. Park JY, Jin KL, Kim DY, Kim JH, Kim YM, Kim KR, et al. Surgical staging and adjuvant chemotherapy in the management of patients with adult granulosa cell tumors of the ovary. Gynecol Oncol. 2012;125(1):80-6.
94. Brown J, Sood AK, Deavers MT, Milojevic L, Gershenson DM. Patterns of metastasis in sex cord-stromal tumors of the ovary: can routine staging lymphadenectomy be omitted? Gynecol Oncol. 2009;113(1):86-90.

95. Morgan RJ, Jr., Armstrong DK, Alvarez RD, Bakkum-Gamez JN, Behbakht K, Chen LM, et al. Ovarian Cancer, Version 1.2016, NCCN Clinical Practice Guidelines in Oncology. Journal of the National Comprehensive Cancer Network : JNCCN. 2016;14(9):1134-63.
96. Zhang M, Cheung MK, Shin JY, Kapp DS, Husain A, Teng NN, et al. Prognostic factors responsible for survival in sex cord stromal tumors of the ovary--an analysis of 376 women. Gynecol Oncol. 2007;104(2):396-400.
97. Homesley HD, Bundy BN, Hurteau JA, Roth LM. Bleomycin, etoposide, and cisplatin combination therapy of ovarian granulosa cell tumors and other stromal malignancies: A Gynecologic Oncology Group study. Gynecol Oncol. 1999;72(2):131-7.
98. van Meurs HS, van der Velden J, Buist MR, van Driel WJ, Kenter GG, van Lonkhuijzen LR. Evaluation of response to hormone therapy in patients with measurable adult granulosa cell tumors of the ovary. Acta Obstet Gynecol Scand. 2015;94(11):1269-75.
99. Sjoquist KM, Martyn J, Edmondson RJ, Friedlander ML. The role of hormonal therapy in gynecological cancers-current status and future directions. Int J Gynecol Cancer. 2011;21(7):1328-33.
100. van Meurs HS, van Lonkhuijzen LR, Limpens J, van der Velden J, Buist MR. Hormone therapy in ovarian granulosa cell tumors: a systematic review. Gynecol Oncol. 2014;134(1):196-205.
101. Brown J, Brady WE, Schink J, Van Le L, Leitao M, Yamada SD, et al. Efficacy and safety of bevacizumab in recurrent sex cord-stromal ovarian tumors: results of a phase 2 trial of the Gynecologic Oncology Group. Cancer. 2014;120(3):344-51.
102. Ud Din N, Kayani N. Recurrence of adult granulosa cell tumor of the ovary: experience at a tertiary care center. Annals of diagnostic pathology. 2014;18(3):125-8.
103. van Meurs HS, Schuit E, Horlings HM, van der Velden J, van Driel WJ, Mol BW, et al. Development and internal validation of a prognostic model to predict recurrence free survival in patients with adult granulosa cell tumors of the ovary. Gynecol Oncol. 2014;134(3):498-504.
104. Karalok A, Turan T, Ureyen I, Tasci T, Basaran D, Koc S, et al. Prognostic Factors in Adult Granulosa Cell Tumor: A Long Follow-Up at a Single Center. Int J Gynecol Cancer. 2016;26(4):619-25.
105. East N, Alobaid A, Goffin F, Ouallouche K, Gauthier P. Granulosa cell tumour: a recurrence 40 years after initial diagnosis. Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC. 2005;27(4):363-4.
106. Hines JF, Khalifa MA, Moore JL, Fine KP, Lage JM, Barnes WA. Recurrent granulosa cell tumor of the ovary 37 years after initial diagnosis: a case report and review of the literature. Gynecol Oncol. 1996;60(3):484-8.
107. Ertas IE, Gungorduk K, Taskin S, Akman L, Ozdemir A, Goklu R, et al. Prognostic predictors and spread patterns in adult ovarian granulosa cell tumors: a multicenter long-term follow-up study of 108 patients. International journal of clinical oncology. 2014;19(5):912-20.
108. Stenwig JT, Hazekamp JT, Beecham JB. Granulosa cell tumors of the ovary. A clinicopathological study of 118 cases with long-term follow-up. Gynecol Oncol. 1979;7(2):136-52.
109. Suri A, Carter EB, Horowitz N, Denslow S, Gehrig PA. Factors associated with an increased risk of recurrence in women with ovarian granulosa cell tumors. Gynecol Oncol. 2013;131(2):321-4.
110. Lee IH, Choi CH, Hong DG, Song JY, Kim YJ, Kim KT, et al. Clinicopathologic characteristics of granulosa cell tumors of the ovary: a multicenter retrospective study. J Gynecol Oncol. 2011;22(3):188-95.
111. Mangili G, Sigismondi C, Frigerio L, Candiani M, Savarese A, Giorda G, et al. Recurrent granulosa cell tumors (GCTs) of the ovary: a MITO-9 retrospective study. Gynecol Oncol. 2013;130(1):38-42.

112. Kimura T, Shiono H, Takemoto T, Ohta Y. Lung metastasis from an ovarian granulosa cell tumor 36 years after the initial diagnosis: report of a case. Surg Today. 2013;43(2):199-202.
113. Sasano S, Yamamoto H, Otsuka T, Imura Y, Nishii N, Nitta S. Pulmonary multiple metastases of ovarian granulosa cell tumor 15 years after initial diagnosis. Jpn J Thorac Cardiovasc Surg. 2000;48(10):655-8.
114. Malmström H, Högberg T, Risberg B, Simonsen E. Granulosa cell tumors of the ovary: prognostic factors and outcome. Gynecol Oncol. 1994;52(1):50-5.
115. Lee YK, Park NH, Kim JW, Song YS, Kang SB, Lee HP. Characteristics of recurrence in adult-type granulosa cell tumor. Int J Gynecol Cancer. 2008;18(4):642-7.
116. Miller BE, Barron BA, Wan JY, Delmore JE, Silva EG, Gershenson DM. Prognostic factors in adult granulosa cell tumor of the ovary. Cancer. 1997;79(10):1951-5.
117. Shim SH, Lee SJ, Kim DY, Kim J, Kim SN, Kang SB, et al. A Long-term follow-up study of 91 cases with ovarian granulosa cell tumors. Anticancer research. 2014;34(2):1001-10.
118. Björkholm E, Silfverswärd C. Prognostic factors in granulosa-cell tumors. Gynecol Oncol. 1981;11(3):261-74.
119. Mangili G, Ottolina J, Gadducci A, Giorda G, Breda E, Savarese A, et al. Long-term follow-up is crucial after treatment for granulosa cell tumours of the ovary. Br J Cancer. 2013;109(1):29-34.
120. Holscher G, Anthuber C, Bastert G, Burges A, Mayr D, Oberlechner E, et al. Improvement of survival in sex cord stromal tumors - an observational study with more than 25 years follow-up. Acta Obstet Gynecol Scand. 2009;88(4):440-8.
121. Leuverink EM, Brennan BA, Crook ML, Doherty DA, Hammond IG, Ruba S, et al. Prognostic value of mitotic counts and Ki-67 immunoreactivity in adult-type granulosa cell tumour of the ovary. J Clin Pathol. 2008;61(8):914-9.
122. Stewart CJ, Doherty D, Guppy R, Louwen K, Leung YC. beta-Catenin and E-cadherin expression in stage I adult-type granulosa cell tumour of the ovary: correlation with tumour morphology and clinical outcome. Histopathology. 2013;62(2):257-66.
123. Färkkilä A, Andersson N, Bützow R, Leminen A, Heikinheimo M, Anttonen M, et al. HER2 and GATA4 are new prognostic factors for early-stage ovarian granulosa cell tumor-a long-term follow-up study. Cancer Med. 2014;3(3):526-36.
124. Mangili G, Ottolina J, Cormio G, Loizzi V, De Iaco P, Pellegrini DA, et al. Adjuvant chemotherapy does not improve disease-free survival in FIGO stage IC ovarian granulosa cell tumors: The MITO-9 study. Gynecol Oncol. 2016;143(2):276-80.
125. Teppo L, Pukkala E, Lehtonen M. Data quality and quality control of a population-based cancer registry. Experience in Finland. Acta Oncol. 1994;33(4):365-9.
126. McConechy MK, Farkkila A, Horlings HM, Talhouk A, Unkila-Kallio L, van Meurs HS, et al. Molecularly Defined Adult Granulosa Cell Tumor of the Ovary: The Clinical Phenotype. Journal of the National Cancer Institute. 2016;108(11).
127. Figo Committee on Gynecologic Oncology T. Current FIGO staging for cancer of the vagina, fallopian tube, ovary, and gestational trophoblastic neoplasia. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2009;105(1):3-4.
128. Engholm G FJ, Christensen N, Kejs AMT, Johannesen TB, Khan S, Leinonen M, Milter MC, Ólafsdóttir E, Petersen T, Stenz F, Storm HH. . NORDCAN: Cancer Incidence, Mortality, Prevalence and Survival in the Nordic Countries, Version 7.3 (07.2016). Association of the Nordic Cancer Registries. Available from http://www-dep.iarc.fr/nordcan.htm.
129. Hemminki E, Kyyronen P, Pukkala E. Postmenopausal hormone drugs and breast and colon cancer: Nordic countries 1995-2005. Maturitas. 2008;61(4):299-304.
130. Cronje HS, Niemand I, Bam RH, Woodruff JD. Review of the granulosa-theca cell tumors from the emil Novak ovarian tumor registry. American journal of obstetrics and gynecology. 1999;180(2 Pt 1):323-7.

131. Roth LM. Recent advances in the pathology and classification of ovarian sex cord-stromal tumors. International journal of gynecological pathology : official journal of the International Society of Gynecological Pathologists. 2006;25(3):199-215.
132. Abell MR. The nature and classification of ovarian neoplasms. Can Med Assoc J. 1966;94(21):1102-24.
133. Gharwan H, Bunch KP, Annunziata CM. The role of reproductive hormones in epithelial ovarian carcinogenesis. Endocrine-related cancer. 2015;22(6):R339-63.
134. Tao X, Zhao N, Jin H, Zhang Z, Liu Y, Wu J, et al. FSH enhances the proliferation of ovarian cancer cells by activating transient receptor potential channel C3. Endocrine-related cancer. 2013;20(3):415-29.
135. Choi JH, Wong AS, Huang HF, Leung PC. Gonadotropins and ovarian cancer. Endocrine reviews. 2007;28(4):440-61.
136. Huhtaniemi I. Are gonadotrophins tumorigenic--a critical review of clinical and experimental data. Mol Cell Endocrinol. 2010;329(1-2):56-61.
137. Ran S, Yu Q, Deng S, Xu L. Luteinizing hormone elevation in ovarian granulosa cell tumor: a case report and review of the literature. J Ovarian Res. 2017;10(1):30.
138. Nasu K, Fukuda J, Yoshimatsu J, Takai N, Kashima K, Narahara H. Granulosa cell tumor associated with secondary amenorrhea and serum luteinizing hormone elevation. International journal of clinical oncology. 2007;12(3):228-30.
139. Negri E, Dal Maso L, Ron E, La Vecchia C, Mark SD, Preston-Martin S, et al. A pooled analysis of case-control studies of thyroid cancer. II. Menstrual and reproductive factors. Cancer Causes Control. 1999;10(2):143-55.
140. Zhu J, Zhu X, Tu C, Li YY, Qian KQ, Jiang C, et al. Parity and thyroid cancer risk: a meta-analysis of epidemiological studies. Cancer Med. 2016;5(4):739-52.
141. Xu L, Port M, Landi S, Gemignani F, Cipollini M, Elisei R, et al. Obesity and the risk of papillary thyroid cancer: a pooled analysis of three case-control studies. Thyroid. 2014;24(6):966-74.
142. Virtanen A, Pukkala E, Auvinen A. Incidence of bone and soft tissue sarcoma after radiotherapy: a cohort study of 295,712 Finnish cancer patients. Int J Cancer. 2006;118(4):1017-21.
143. Travis LB, Holowaty EJ, Bergfeldt K, Lynch CF, Kohler BA, Wiklund T, et al. Risk of leukemia after platinum-based chemotherapy for ovarian cancer. N Engl J Med. 1999;340(5):351-7.
144. Mellemkjaer L, Friis S, Olsen JH, Scelo G, Hemminki K, Tracey E, et al. Risk of second cancer among women with breast cancer. Int J Cancer. 2006;118(9):2285-92.
145. Lambe M, Hsieh CC, Chan HW, Ekbom A, Trichopoulos D, Adami HO. Parity, age at first and last birth, and risk of breast cancer: a population-based study in Sweden. Breast cancer research and treatment. 1996;38(3):305-11.
146. Layde PM, Webster LA, Baughman AL, Wingo PA, Rubin GL, Ory HW. The independent associations of parity, age at first full term pregnancy, and duration of breastfeeding with the risk of breast cancer. Cancer and Steroid Hormone Study Group. Journal of clinical epidemiology. 1989;42(10):963-73.
147. Gherman RB, Parker MF, Macri CI. Granulosa cell tumor of the ovary associated with antecedent tamoxifen use. Obstetrics and gynecology. 1994;84(4 Pt 2):717-9.
148. Tanaka T, Kato T, Ohmichi M. Granulosa cell tumor of the ovary after long-term use of tamoxifen and toremifene. The journal of obstetrics and gynaecology research. 2012;38(12):1379-84.
149. Schiavon G, Smith IE. Status of adjuvant endocrine therapy for breast cancer. Breast cancer research : BCR. 2014;16(2):206.
150. Ottolina J, Ferrandina G, Gadducci A, Scollo P, Lorusso D, Giorda G, et al. Is the endometrial evaluation routinely required in patients with adult granulosa cell tumors of the ovary? Gynecol Oncol. 2015;136(2):230-4.

151. Huang BS, Sun HD, Hsu YM, Chang WH, Horng HC, Yen MS, et al. Clinical presentation and outcome of adult-type granulosa cell tumors: a retrospective study of 30 patients in a single institute. J Chin Med Assoc. 2014;77(1):21-5.
152. Kim YM, Jung MH, Kim KR, Kim JH, Kim YT, Nam JH, et al. Adult granulosa cell tumor of the ovary: 35 cases in a single Korean Institute. Acta Obstet Gynecol Scand. 2006;85(1):112-5.
153. Nosov V, Silva I, Tavassoli F, Adamyan L, Farias-Eisner R, Schwartz PE. Predictors of recurrence of ovarian granulosa cell tumors. Int J Gynecol Cancer. 2009;19(4):628-33.
154. Sehouli J, Drescher FS, Mustea A, Elling D, Friedmann W, Kuhn W, et al. Granulosa cell tumor of the ovary: 10 years follow-up data of 65 patients. Anticancer research. 2004;24(2C):1223-9.
155. Petrillo M, Ferrandina G, Fagotti A, Vizzielli G, Margariti PA, Pedone AL, et al. Timing and pattern of recurrence in ovarian cancer patients with high tumor dissemination treated with primary debulking surgery versus neoadjuvant chemotherapy. Ann Surg Oncol. 2013;20(12):3955-60.
156. Creasman WT. Hormone replacement therapy after cancers. Curr Opin Oncol. 2005;17(5):493-9.
157. Guidozzi F. Estrogen therapy in gynecological cancer survivors. Climacteric. 2013;16(6):611-7.
158. Guidozzi F, Daponte A. Estrogen replacement therapy for ovarian carcinoma survivors: A randomized controlled trial. Cancer. 1999;86(6):1013-8.
159. Li L, Pan Z, Gao K, Zhang W, Luo Y, Yao Z, et al. Impact of post-operative hormone replacement therapy on life quality and prognosis in patients with ovarian malignancy. Oncol Lett. 2012;3(1):244-9.
160. Li D, Ding CY, Qiu LH. Postoperative hormone replacement therapy for epithelial ovarian cancer patients: a systematic review and meta-analysis. Gynecol Oncol. 2015;139(2):355-62.
161. Eeles RA, Tan S, Wiltshaw E, Fryatt I, A'Hern RP, Shepherd JH, et al. Hormone replacement therapy and survival after surgery for ovarian cancer. BMJ. 1991;302(6771):259-62.
162. Mascarenhas C, Lambe M, Bellocco R, Bergfeldt K, Riman T, Persson I, et al. Use of hormone replacement therapy before and after ovarian cancer diagnosis and ovarian cancer survival. Int J Cancer. 2006;119(12):2907-15.
163. Ursic-Vrscaj M, Bebar S, Zakelj MP. Hormone replacement therapy after invasive ovarian serous cystadenocarcinoma treatment: the effect on survival. Menopause. 2001;8(1):70-5.